Strategic Control, Consolidation and Poly-drug use; the Relative Contributions to Verbal Memory Impairment in Recreational Ecstasy Users

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Abstract

Tests of verbal learning and memory have frequently been used to examine memory performance in frequent 3,4-methylendioxymethamphetamine ("ecstasy") consumers, and meta-analyses have identified verbal learning and memory as the cognitive domain most adversely affected by regular ecstasy use (eg. Kalechstein, et al., 2007; Zakzanis, Campbell & Jovanovski, 2007). Tests of verbal memory are diverse however and include prose recall, multi-trial free recall and recognition tests. As such, it is unclear which specific measures of verbal learning and memory are most impaired by ecstasy use. Ascertaining which measures are most affected by chronic ecstasy use can inform as to which neural networks may be more likely candidates for explaining ecstasy related cognitive impairments. Study 1 therefore aimed to clarify which measure was most impacted by ecstasy use, by separating the domain of verbal learning and memory into three dependent variables that are commonly reported in the ecstasy and memory literature; Trial 1, Total and Delayed recall. This meta-analysis showed that ecstasy use does not impact on these three measures equally, with only a trivial effect of ecstasy use (g = -.15) found for Trial 1 (immediate recall), and moderate magnitude effects for Total (g = -.71) and Delayed recall scores (g = -.67) scores.

The *Working with Memory* model (Moscovitch & Winocur, 1992; Moscovitch & Winocur, 2002; Moscovitch, 2008) posits that networks in the prefrontal cortex work with the hippocampus to organise information to enhance encoding and retrieval efficiency. Using this model as a framework, Study 2 sought to examine the relative contributions of frontal and hippocampal cognitive processes contributing to the Total and Delayed recall deficits for ecstasy and poly-drug users. This was achieved by recruiting regular consumers of ecstasy only (n = 15), cannabis only (n = 17) regular consumers of both ecstasy and cannabis (n = 20) and drug naïve participants (n = 17). Participants completed two multi-trial word learning tasks; one of which consisted of non-related words and the other comprised of semantically related words. Indices of semantic and subjective clustering were calculated to ascertain the involvement of frontal/strategic processes to memory scores, as well as several non-traditional memory indices that specified which words were gained and lost between list learning trials and assessed patterns of forgetting over multiple trials. Results showed moderate to large magnitude effects of ecstasy

use for traditional measures such as Total recall (unrelated list; *g* = -.92, related list; *g* = -1.10) and Delayed recall (unrelated list only; *g* = -1.02) and these effects were independent of the effects of cannabis and other drug use. A clear inter-trial consolidation deficit was also apparent for ecstasy users, who lost more words between each learning trial than non-users (*g* = -.67) however ecstasy users did not make fewer gains relative to the other drug groups. The lack of difference in gains suggests intact acquisition/encoding for ecstasy users, however the higher number of "losses" on the subsequent trial indicate these gains were not able to be consolidated and hence were "lost." This poor consolidation was also evident on the measures of forgetting, with ecstasy users more likely to forget words that had previously been recalled once (*g* = .73) and twice (*g* = -.63) compared with non-users. Strategic organisation of to-be-remembered words were also associated with moderate to large magnitude effects for ecstasy (*g* = -.53 for the unrelated list and *g* = -.97 for the related list) and not cannabis use, implicating deficient engagement of the prefrontal cortex as a contributor to verbal memory deficits associated with ecstasy use.

In Study 3, two source memory tasks which differed in degree of difficulty were used to assess source memory performance for ecstasy users for the first time. Source judgements require the specific recollection of the context in which the encoding occurred, such as the colour a word was presented in. Poor source judgements have been observed in drug-naïve volunteers during acute tryptophan depletion, suggesting episodic recollection processes may be sensitive to reduced 5-HT functioning (McAllister-Williams, Massey & Rugg, 2004). The pattern of results for ecstasy users resembled source judgements of older adults, with no differences apparent on the easier version of the task between drug groups, however a significant moderate ecstasy related effect was observed for the more difficult, strategic version of the task (*g* = -.57). This result was thus consistent with the previous finding that ecstasy users are deficient in strategic processes that assist memory performance, and also suggest that source binding associated with the hippocampus may be impaired by ecstasy exposure.

With regard to poly-drug use, this thesis found limited evidence for an effect of cannabis use on the various memory measures, with one notable exception; the number of specific words lost between the final learning trial and delayed recall was significantly greater for both cannabis and ecstasy users, suggesting the often

reported Delayed recall deficit for ecstasy users may be confounded by concomitant cannabis use.

Overall, the current thesis has found significant strategic/organisational deficits which are associated with impaired prefrontal functioning, *as well as* consolidation and source memory deficits associated with hippocampal dysfunction for regular ecstasy users, and these deficits were best accounted for by ecstasy rather than poly-drug use. As such, conclusions that verbal memory deficits reported for regular consumers of ecstasy are primarily a consequence of poly-drug use are at present, premature.

Table of Contents

List of Tables

List of Figures

Chapter 1.

The Pharmacology, Patterns of Use, Acute and Long-term Effects of Ecstasy

"Ecstasy" is a popular recreational drug in various countries, and is the second most used illicit drug in Australia. For the preceding twenty years however, there has been an accumulation of evidence suggesting that MDMA, the main psychoactive ingredient of "ecstasy" can cause changes to the serotonin system in humans. In addition to serotonergic alterations, ecstasy use is often associated with cognitive deficits, the most prominent being deficits in verbal memory recall. In this thesis, these verbal memory deficits are further examined in a sequence of studies that aim to better understand the global, as well as more specific cognitive components of memory that are most affected by ecstasy use.

Chemical classification of MDMA

MDMA is an abbreviation of 3,4-methylenedioxymethamphetamine, which is the main psychoactive ingredient in the popular illicit drug, 'ecstasy'. MDMA is a ring substituted amphetamine derivative that is structurally related to mescaline, amphetamine and to the monoamine neurotransmitters (Capela et al., 2009). In humans, MDMA is well absorbed when taken orally, and studies of humans following ingestion of MDMA show maximum concentration appears at 1.5 – 3 hours post consumption (de la Torre et al., 2000; Farre et al., 2004). In the present thesis, the term 'MDMA' will be used to refer to pure MDMA administered in research settings and the term 'ecstasy' will be used to refer to tablets purchased by regular ecstasy consumers.

Current use of ecstasy in Australia

According to the 2010 National Drug Strategy Household Survey Report, after cannabis, ecstasy is the second most commonly used illicit drug in Australia, with 3.0% of people aged 14 years or older using ecstasy in the previous 12 months in 2010. Among 18-19 year olds, 9.8% reported having ever used ecstasy, and 6% had used it in the last 12 months. Ecstasy use was highest among people aged between 20–29 years, with 24.2% of participants reporting they had used ecstasy, and 9.9% reporting having used ecstasy in the last 12 months.

An annual survey of 574 regular ecstasy users across Australia (Ecstasy and Related Drugs Reporting System-EDRS) reported that people who use ecstasy are typically young (mean age of 24) heterosexual (88%) and a higher proportion of recreational ecstasy consumers are male (69%). They are typically tertiary educated (46%) or in part time or full time employment, with minimal contact (5%) with drug treatment programs. Regular ecstasy users preferred to swallow ecstasy in tablet form on occasional weekends at nightclubs or pubs. The median number of tablets taken per session of use was two. People who regularly take ecstasy also regularly use other drugs, with 98% of the sample having used alcohol in the preceding six months, 85% had used cannabis during this time and 60% had used some form of methamphetamine (Sindicich & Burns, 2011).

Patterns of use and purity of ecstasy in Tasmania

Due to the variation in ecstasy purity and patterns of use between cities and over time, it is important to consider the characteristics of ecstasy use within the region where the research sample was acquired. The data presented later in this thesis was collected from regular ecstasy users from Hobart, Tasmania during 2008-2009. In 2009, the Tasmanian EDRS interviewed 100 ecstasy users who had used ecstasy fortnightly on average during the preceding six months. They had all used ecstasy in tablet form and their preferred route of administration was swallowing. Consistent with trends nationally, these participants used a median of two ecstasy pills per session. Poly drug use was also the norm; during the six months preceding the interview 99% of the sample had used alcohol, 76% of the sample had used cannabis and 46% had used methamphetamine in powder form.

Ecstasy seizures are reported under "phenethylamines" seizures in the Illicit Drug Data Report (IDDR, 2008-2009) although the majority of seizures analysed for purity were ecstasy related. Not all ecstasy seizures were analysed, so the purity data is not representative and only provide an indication of ecstasy purity in Australia. Between 2007 and 2009, the median purity in Tasmania remained stable at approximately 25% and the Tasmanian EDRS sample of 2009 usually reported that ecstasy purity was "medium" and had remained so for the preceding six months. In a review of the purity of ecstasy tablets internationally, Parrott (2004) demonstrated

that almost all ecstasy tablets contain MDMA as their active ingredient and this is consistent with testing of Australian police seizures (Quinn, 2009).

The effects of MDMA administration

MDMA affects peripheral and central nervous system functions mainly via the monoaminergic system. Several studies in rats have shown that MDMA inhibits monoamine oxidase, the enzyme which is responsible for the breakdown of serotonin, resulting in a reduction of metabolic breakdown. MDMA also inhibits tryptophan hydroxylase, the rate limiting enzyme for serotonin synthesis (Cadet, Jayanthi & Lyles, 2007). At the serotonergic synapse and neuronal terminal, MDMA interacts with the serotonin transporter (SERT) to enter the neuronal terminal and elicit an acute and rapid release of up to 80% of stored serotonin from the presynaptic neurons, almost depleting the vesicular neurotransmitter stores (see Figure 1.1). Through its interactions with SERT, MDMA increases the extracellular concentration of serotonin in multiple brain regions and also releases smaller amounts of dopamine and norepinephrine (Green et al., 2004; Lyles & Cadet, 2003; Yamamoto, Nash, & Gudelsky, 1995). Microdialysis experiments in rats show that MDMA increases extracellular serotonin and dopamine levels in the brain, with the effects on serotonin having the greatest magnitude (Gudelsky & Nash, 1996; Yamamoto & Spanos, 1988). MDMA has an affinity for several serotonin receptors, including, the activation(5-$HT2_A$, 5-$HT1_B$/1_D) or inhibition (5-$HT2_C$) of which are believed to underlie the subjective, behavioural and neurochemical reactions associated with MDMA exposure (Cadet, Jayanthi & Lyles, 2007; Fletcher, Korth, Robinson & Baker, 2002). MDMA also has a high affinity of similar magnitude to that of SERT, for brain nicotinic and acetylcholine receptors which may account for cholinergic neurotransmission alterations associated with ecstasy use (Cadet et al.).

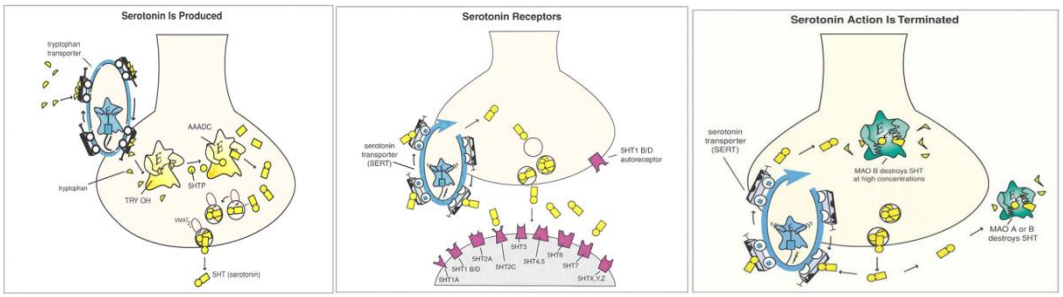

Figure 1.1. Functioning of the serotonergic system (Stahl, 2008). Serotonin is produced in a pre-synaptic neuron and then taken up by synaptic vesicles (left). When the neuron is activated, serotonin is released into the synaptic cleft where it binds to serotonin receptors on a post-synaptic neuron (centre). Excess serotonin is cleared from the synaptic cleft by the serotonin transporter, which releases serotonin out of the synapse and back into the pre-synaptic neuron. Alternatively, excess serotonin is broken down by enzymes such as monoamine oxidase (right).

Acute effects of MDMA on humans

Typical recreational doses of MDMA range from 50-150mg, and several studies have administered these doses (usually 1.5mg per kilogram) to healthy volunteers to examine the acute effects of ecstasy. MDMA increases intracellular serotonin and dopamine levels for several hours, leading to pleasurable subjective effects as well as increased stress on the immune, metabolic and cardiovascular systems, and changes to regional cerebral blood flow (De La Torre et al., 2000).

When given in typical recreational doses to human participants, double-blind placebo controlled studies have demonstrated that MDMA increases cardiovascular activity, including heart rate and blood pressure, which peak 1-2 hours post MDMA consumption (De La Torre et al., 2000a, Downing, 1986; Freedman et al., 2005; Grob et al., 1996; Lester et al., 2000; Mas et al., 1999; Vollenweider et al., 1998;) and continue to be observed four hours post MDMA consumption (Gouzoulis et al., 1993. Gouzoulis-Mayfrank et al., 1999). MDMA administration has also caused changes to regional cerebral blood flow, with increases reported for ventromedial frontal and occipital cortex, inferior temporal lobe and cerebellum, and a decrease in the motor and somotasensory cortex and temporal lobes (Gamma, Buck, Berthold, Liechti, & Vollenweider, 2000).

The same doses have been shown to result in neuroendocrine changes, such as increased plasma levels of oxytocin, vasopressin, corticotropin, dehydroepiandrosterone and a reduction in sodium concentrations (de la Torre et al., 2000; Harries et al., 2002; Wolff et al., 2006). The most consistently reported reductions are found in cortisol and prolactin levels (Dumont & Verkes, 2006). The hypothalamic–pituitary–adrenal axis secretes cortisol throughout the day and this secretion is increased during times of increased stress. MDMA (0.5mg) has been demonstrated to increase cortisol levels to 100% above baseline in the laboratory and this effect appears to be increased to around 800% over baseline when MDMA is taken during dance clubbing, which may be due to increased physiological stress as a result of the combined effects of prolonged dancing, higher ambient temperature, loud music and dehydration (Cole & Sumnall, 2003; Parrott, 2002; Parrott, Lees, Garnham, Jones, Wesnes, 2006; Parrott, Lock, Conner, Kissling & Thome, 2008). The chronic release of cortisol via the HPA suppresses hippocampal functioning and has been demonstrated to negatively impact cognition, in particular episodic memory (Geoffrey, Hertzman, Li & Power, 2012; Herbert et al., 2006).

The increase in MDMA mediated cortisol release has also been shown to induce immunosuppression and thus lead to impaired immunocompetence (Pacifici et al., 2001; Connor, 2004; Connor et al., 2005). Immunocompetence is the ability of an organism to react defensively against antigens. Nelson et al. (2008) demonstrated reduced immunocompetence in MDMA treated mice compared with drug free controls after being infected with a pathogen. MDMA consumption appears to be followed by a period of impaired immunocompetence, leading people who use ecstasy to have an increased susceptibility to infection.

Acute MDMA intoxication is also associated with inappropriate antidiuretic hormone secretion and hyponatraemia (low concentrations of sodium ions in the blood) which can be brought on by excessive fluid intake as a result of increased temperature and physical activity (Cole & Sumnall, 2003; Parrott, 2002). This condition can be exacerbated due to MDMA induced urinary retention (Delagdo, Caruso, Waksman, Hongiman & Stillman, 2004; Inman & Greene, 2003; Bryden, Rothwell & O'Reilly, 1995).

Another acute effect of MDMA use is hyperthermia, which may be one of the mechanisms by which changes to the serotonergic system occur after repeated ecstasy use (eg. Parrott, 2001; 2004; 2006). Although non-human studies previously indicated that MDMA-induced hyperthermia was dependent on ambient temperature (Green et al., 2004) several studies have reported a modest increase in body temperature (between 0.2-0.3 degrees Celsius) in human volunteers post MDMA administration, regardless of ambient temperature (eg. Ciohen & Cocolres, 1997; Kolbrich et al., 2008; Vollenweider et al., 1998). In one study (Freedman et al, 2005), human volunteers were administered MDMA under climate controlled conditions in ambient temperatures of 18 and 30 degrees Celsius. MDMA consumption was shown to increase core body temperature by 0.3 and 0.6 degrees respectively, and the authors suggested these elevations were likely due to the 50-100% increase in metabolic rate (Freedman, Johanson & Tancer, 2005). Further, ecstasy users often don't report increase in body temperature or heart rate as uncomfortable (Capela, 2009) which may result in prolonged physical activity, thus increasing the risk of poor thermoregulation and changes in metabolic rate. These factors can combine to increase oxidative stress, which has been suggested to exacerbate serotonergic cellular damage during the acute phase of MDMA intoxication (Miranda et al., 2007).

To account for the variations of symptoms associated with regular ecstasy use, Parrott (2006) postulated a bioenergetic stress model of MDMA . This model predicts that drug related factors such as disrupted thermoregulation, acute neurotransmitter release, oxidative stress, cortisol release and co-use of other drugs interact with non-drug factors such as the environment in which time is spent intoxicated, ambient temperature, physical activity, and other factors such as immune-competence, sleep and genetics. For example, the increase in cardiovascular activity as a result of dancing could contribute to MDMA induced cortisol release and hyperthermia. Immunocompetence may be additionally impaired by becoming dehydrated while on the drug and due to loss of appetite and sleep in the following days (Pacifici, et al., 2002; Connor, 2004). This model therefore partially accounts

for discrepancies in ecstasy related findings by considering various factors other than MDMA itself that can contribute to the sequale of regular ecstasy use.

Subjective effects of MDMA

The most frequently reported subjective effects of MDMA intoxication within the first 24 hours include improved mood, increased confidence and energy, increased social interaction, euphoria, warmth, friendliness, feeling thirsty, heightened perception of sound, colour, touch and increased heart rate (Cohen & Cocores, 1997; Davison & Parrott, 1997; Verheyden, Henry & Curran, 2003; Vollenweider et al., 1998). With increasing duration of ecstasy use, reports of positive effects decline (Murphy, Waring & Fisk. 2006) and common subjective experiences include becoming drug tolerant, poor concentration and feeling depressed (Verheyden et al., 2003). Subjective effects have been demonstrated to be related to the pharmacological content of tablets sold as ecstasy, as assessed by self-reports by regular ecstasy users who handed in their tablets for chemical analysis. MDMA dose was positively related to positive effects (Odds ratio, 1.01, 95% CI: 1.009 – 1.014, $p < .001$) and logistic regression with different doses as the categorical variable further showed that the probability of experiencing positive effects increased until 81 – 100mg of MDMA and then slowly decreased with higher doses of MDMA. The probability of experience adverse effects rapidly increased at doses higher than 120mg (Brunt , Koeter, Niesink & Van den Brink, 2012). MDMA releases serotonin approximately 10 times more potently than methamphetamine, and releases dopamine approximately six times less potently than methamphetamine (Sarkar & Schmued, 2010) and subjective effects are consistent with this variation in neurotransmitter release, with increased empathy and feeling close to others being specific to MDMA, whereas subjective effects of dopaminergic drugs such as methamphetamine and cocaine appear to be related to the mesolimbic pathway, and include feeling a rush, liking the drug, craving the drug, and later an experience of "crashing" (Walsh, Stoops, Moody, Lin & Bigelow, 2009).

In summary, MDMA is a popular illicit drug in Australia, with nearly a quarter of all illicit drug users having used it in Australia in 2010. Although the amount of MDMA sold as ecstasy in Australia varies, police seizure evidence indicates that pill purity was relatively stable in Tasmania in 2008-2009, which was

the period of recruitment for this study. The subjective effects of ecstasy, including euphoria, increased empathy and increased confidence probably arise as a consequence of its action on the brain serotonin, and to a lesser extent dopamine. MDMA has also been demonstrated to cause neuroendocrine alterations including increased cortisol and prolactin release, as well as hyperthermia, increased cardiovascular activity in humans. Although some of these acute effects are a consequence of MDMA's actions on neurotransmitters, variations in symptoms, and in particular, discrepant reports of ecstasy related memory deficits, may be due to interactions between drug and non-drug factors such as poly-drug use, genetics, increased physical activity, dehydration or excessive fluid intake, and higher cortisol levels during time spent on the drug.

Chapter 2

The Prevalence and Effects of Cannabis use amongst Regular Ecstasy Users

The use of cannabis in Australia

The 2010 National Drug Strategy Household Survey Report found cannabis to be the most commonly used illicit drug in Australia. Of people over the age of 14, 35.4% reported having ever used cannabis, and 10.3% had used cannabis in the past 12 months. For the preceding 12 months, 23.1% of people aged between 18 and 19, and 25% of persons aged between 20 and 29 had used cannabis. Amongst frequent (at least monthly) ecstasy users in Australia in 2011, 31% had also used cannabis in the previous month, and 85% had used cannabis in the preceding six months (Sindicich & Burns, 2011). A high percentage (49%) of these 'regular' ecstasy users used cannabis and ecstasy concomitantly, and 58% reported using cannabis when coming down from a heavy session of ecstasy. These high rates of concomitant ecstasy and cannabis use are not always adequately controlled for in the ecstasy literature (Curran, 2000; Parrot, 2006) and the present thesis attempts to address this by including a Cannabis-only group in the design as an independent variable. As such, a brief review of the neuroanatomical and neuropsychological effects of cannabis use follows.

Acute effects of cannabis

Cannabis is a plant derived illicit drug containing 70 types of cannabinoids, which interact with endogenous cannabinoid receptors in the human body. The most potent psychoactive cannabinoid is Δ^9-tetrahydrocannabinol (THC). Neuronal cannabinoid receptors CB1 and CB2 have been found in rat, guinea pig, dog, monkey, pig and human brains. CB1 is found on presynaptic terminals and modulates neurotransmission through inhibiting adenylyl cyclase and modulating adenosine 3′,5′-cyclic monophosphate (a metabolic regulator) production (McAllister & Glass, 2002; Pacher, Batkai & Kunos, 2006). The distribution of CB1 is mostly localised to the CNS, including the cerebral cortex, hippocampus, amygdala, basal ganglia, cerebellum, thalamus and brainstem. CB2 receptors are mostly present in some immune cells (Ashton, 2001). In non-human animals, consumption of THC results in binding at CB1 and CB2, and increases the release of

dopamine from the nucleus accumbens and prefrontal cortex. The increase in dopamine is believed to cause some of the pleasant subjective acute effects of THC consumption in humans (Green, 2006) such as euphoric mood, relaxation, increased sociability and enhanced sensory perception (Ashton, 2001; Green, 2003). The acute effects of cannabis begin within a few minutes after smoking the drug, and usually diminish within four hours (Pacher et al., 2006).

Functional and structural effects of cannabis

Martin-Santos et al. (2010) conducted a review of the neuroimaging literature on the acute and chronic effects of cannabis on brain structure and function. The 41 studies which met criteria for the review comprised 655 cannabis users and 402 controls. Chronic cannabis users were defined as having used cannabis several times per week for a minimum of two years, and recreational users were defined as using up to four times per month. The control participants had less than 15 occasions of use in their lifetime. The most consistent results came from studies that examined the acute effects of experimental THC or marijuana cigarette administration on brain activity. Three PET studies reported global increases in regional cerebral blood slow (rCBF) for recreational and chronic cannabis users following smoking a marijuana cigarette (containing THC) compared with baseline or placebo conditions. Of the six studies that examined the effects of experimental administration of THC on resting state, all reported global increases in rCBF for cannabis users relative to controls, and some studies reported subjective levels of intoxication were positively correlated with increases in anterior and posterior rCBF (Matthew et al., 2002) and also with increases in the anterior cingulate cortex (Matthew et al., 1997) frontal cortex (Matthew et al., 1999) and in the cerebral cortex (Volkow et al., 1996). These results were consistent for recreational and chronic cannabis users following smoking a marijuana cigarette (Matthew et al., 1992; Matthew & Wilson, 1993).

Studies that compared resting brain activity between chronic cannabis users and controls in the absence of THC administration reported resting global, prefrontal and anterior cingulate cortex blood flow was lower in cannabis users (maximum abstinence was two weeks) compared with controls (eg. Block et al., 2000; Lundqvist et al., 2001) although Sneider et al., (2008) examined changes in regional

cerebral blood volume (rCBV) between chronic cannabis users and controls and reported increased rCBV in the right frontal, temporal lobes (bilaterally) and the cerebellum at 6 hours, 36 hours and 7 days abstinence from cannabis. After 28 days of monitored abstinence, cannabis users continued to show this altered neural activity with the exception of the frontal region, where the activation was no longer different from controls, suggesting that the frontal cortex begins to normalise with continued cannabis abstinence.

Structural differences between cannabis users and controls have been examined using various MRI techniques. One study used voxel-based morphemetry to examine possible brain structural differences in a group of 11 chronic cannabis users and 8 controls, and reported reduced grey matter volume in bilateral hippocampus (Matochik, Eldreth, Cadet & Bolla, 2005). Similarly, Yucel et al. (2008) reported bilateral reduction in the hippocampal and amygdala areas, with the hippocampal volume reduction being negatively related to cumulative exposure to cannabis over the preceding ten years. The participants in this study were carefully selected on the basis of prior exposure, all had been using daily for a minimum of ten years (mean = 19.7 years) and smoked a minimum of five marijuana cigarettes a day. Hermann et al. (2007) used MR spectroscopy to examine various brain regions in regular cannabis users and controls and reported diminished axonal integrity for cannabis users in the dorsolateral prefrontal cortex, however the cannabis users in this study were not required to undergo a required period of abstinence, thus these effects could be acute rather than residual.

In an MRI which compared hippocampal volumes between adolescents who used either alcohol and cannabis, alcohol only or who were drug free, only those with a history of alcohol use alone had smaller hippocampal volumes (Medina et al., 2007). Most MRI studies that have used region of interest analyses have failed to identify structural brain changes in regular cannabis users compared to controls (Block et al., 2000; DeLisi et al., 2006; Gruber & Yurgelun-Todd, 2005; Tzilos et al., 2005; Jager et al., 2007). Overall, the studies that have reported structural differences associated with cannabis use had small sample sizes, used very heavy, atypical cannabis users or had no required abstinence period, and as such more

research is required to investigate cannabis induced structural alterations in the human brain.

Evidence for the effects of cannabis on human dopaminergic functioning

Although animal studies have frequently reported increases in CNS dopamine release following cannabis consumption, dopaminergic fluctuations as a consequence of regular cannabis use in humans are reported inconsistently in the literature thus far. A few recent PET studies have used a selective dopamine D_2/D_3 tracer to examine the effects of THC administration on dopamine receptor binding. Stokes, Mehta, Curran, Breen and Grasby (2009) reported an increase in psychosis-like symptoms in participants with cannabis experience following experimental administration of THC at a dose equivalent to a standard marijuana cigarette, however compared to placebo there were no significant differences in dopamine receptor binding in the striatum. A SPECT study administered a relatively low dose of THC (2.5mg, compared with the usual 10mg) intravenously, and at this dose there was still an increase in positive type symptoms of schizophrenia, but no differences in receptor binding in the caudate or putamen relative to placebo (Barkus, et al., 2010). Sevy et al. (2008) examined differences in dopamine receptor availability between currently abstinent cannabis dependent persons (minimum of twelve weeks abstinence) and drug naïve controls. There were no differences in D_2 and D_3 receptor availability between groups, although the cannabis group did have lowered glucose metabolism in the orbitofrontal cortex and bilateral putamen. Bossong et al. (2009) however, examined the effects of THC inhalation on seven drug naïve participants and reported decreased D_2 and D_3 binding in the ventral striatum and dorsal putamen, indicating that an increase in dopamine release had occurred within these regions. Using PET with a specific dopamine transporter (DAT) radiogland, Leroy et al. (2011) assessed DAT activity in tobacco dependent and marijuana dependent participants in comparison with non-smokers. Region of interest analysis reported a 20% decrease in DAT availability in the dorsal striatum in smokers compared with controls, and whole brain analysis showed lower DAT availability in the ventral striatum, midbrain and thalamus. Although DAT availability was lower in cannabis dependent compared with tobacco dependent persons, this difference did not reach statistical significance. Further, DAT availability in the associative striatum has

been demonstrated to be related to earlier onset or longer duration of use in persons with mild to moderate cannabis dependence (Urban et al., 2012) , and this relationship has also been reported for the neurocognitive data also, with greater cognitive deficits associated with earlier onset of use (Pope et al., 2003). It therefore appears as though alterations to dopaminergic neurotransmission in cannabis users are mediated by age of onset.

Ecstasy and cannabis are frequently used together, and their combined effects may be interactive rather than additive (Croft et al., 2001; Fisk, Montgomery, Wareing & Murphy, 2006; Parrot, Gouzouikis-Mayfrank, Riodgers & Solowij, 2004). It is therefore useful to consider the influence of cannabis when investigating the cognitive effects of ecstasy use. Cannabis receptors are distributed throughout the human brain, including the cerebral cortex and hippocampus. Cannabis intoxication increases the release of dopamine from the nucleus accumbens and prefrontal cortex, however evidence that cannabis use is associated with alterations to DAT density and availability in the prefrontal cortex and hippocampus is limited at this time. Cannabis is a particularly useful drug to compare with ecstasy as each drug is associated with greater, though not exclusive, release of dopamine and serotonin respectively, and this difference means that the differential effects of each drug on the memory outcomes in the current thesis can be attributed with some degree of confidence to either the serotonergic or dopaminergic systems. Further, since there is still debate surrounding ecstasy specific, as opposed to poly-drug effects on verbal memory, including a Cannabis-only using group, and comparing their performance with an Ecstasy-only group and an Ecstasy-plus cannabis group can better address the role of poly-drug use in memory deficits for ecstasy users.

Chapter 3
The Effects of Repeated MDMA Exposure on the Human Serotonergic System

As discussed previously, typical recreational doses of MDMA releases up to 80% of stored serotonin from presynaptic neurons in humans, increasing the extracellular concentration of serotonin in multiple brain regions (Green et al., 2004). Serotonin (5-HT) is a biogenic amine which acts as a neurotransmitter extensively throughout the central (CNS) and peripheral nervous systems. The neuropsychological and behavioural processes modulated by serotonin include mood, perception, appetite, sleep wake cycle, attention and memory (Berger, Gray, Bryan & Roth, 2009; Rubenstein, 1998). In rats, non-human primates and humans, the most notable alterations in MDMA induced neurotransmission involve the serotonergic system (Sarkar & Schmued, 2010). Because of these alterations to serotonergic functioning, the last two decades has seen an influx of reports claiming that ecstasy has the ability to produce *neurotoxic* effects in laboratory animals and humans. The meaning of the term neurotoxicity has received little attention in the literature until relatively recently, when the lack of consensus regarding its definition was raised by Biezonski and Meyer (2011) who suggested there has been a lack of differentiation between substances that cause a shortage of serotonin markers within intact neurons, and those that induce irreparable neurodegeneration. In the current thesis, the term neurotoxicity is reserved for when authors have specifically used this term to describe the effects of MDMA on the serotonergic system. Further, since neurotoxicity can be defined as an adverse change in brain structure or function which may be reversible or permanent, the term *serotonergic alterations* and synonyms are used to acknowledge the presence of serotonergic abnormalities in ecstasy users and also the current lack of understanding as to whether these are transient or permanent.

Overview of the serotonergic system

The cell bodies of serotonin neurons are located in the raphe nuclei a collection of neurons localised along the rostrocaudal midline of the brain stem. The dorsal and median raphe nuclei contain approximately 85% of serotonin neurons found in the brain (Barnes & Sharpe, 1999; Berger et al., 2009; Hornung &

deTribolet, 1995). Axons originating in the dorsal raphe nuclei project to the ventral hippocampus, amygdala, lateral septum, striatum and prefrontal and cerebral cortex. The dorsal hippocampus and cerebral cortex are also innervated by the median raphe nuclei (see Figure 1.2). The frontal cortex receives innervations that are twice as dense as those to the parietal and occipital lobes, with the dorsal frontal cortex containing the most serotonergic neurons (Barnes & Sharp, 1999; Hornung, 2003; Hoyer et al., 1994; Michelsen, Schmitz & Steinbusch, 2007).

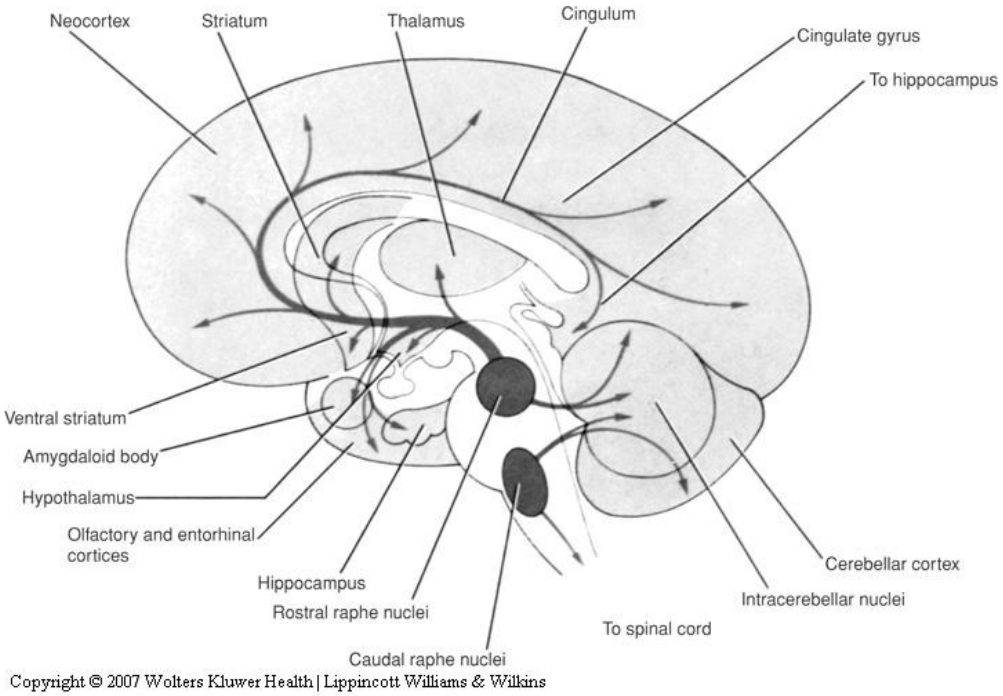

Figure 1.2. Serotonergic pathways (represented by arrows) in the human brain.

Based on their morphology and origin, serotonergic axons differ in width and shape. Fine varicose axons originate from the dorsal raphe nuclei and are distributed through all cerebral cortical areas, but are more concentrated in layer IV of cortex and in primary sensory areas (Hornung, Fritschy & Tork, 1990; Wilson & Molliver, 1991). The thicker axons stem from the medial raphe nuclei and are restricted to

layers of the hippocampus and the medial and frontal neocortex. In rats and non-human primates, acute doses of MDMA preferentially affect the fine axons originating from the dorsal raphe nuclei, leaving the thicker axons intact (Mamounas, Mullen, O'Hearn & Molliver, 1991; O'Hearn, Battaglia, DeSouza, Kuhar & Molliver, 1988) although Gartside McQuade and Sharpe (1998) failed to observe differential reactions from dorsal and medial originating axons after an acute dose of MDMA in rats, concluding that MDMA affected the axons equally. MDMA also appears to differentially alter the neural plasticity of axons following MDMA exposure. The regeneration of serotonin axons after MDMA exposure may depend on their proximity to the raphe nuclei, with axon terminals in the frontal cerebral cortex showing lasting enervation, and those closer to the raphe nuclei showing greater re-innervation in squirrel monkeys (Fischer, Hatzidimitriou, Katz & Ricaurte, 1995).

Serotonin effects neural activity via excitation or inhibition of approximately 14 different 5-HT receptor subtypes (Barnes & Sharp, 1999; Hoyer, Clarke et al., 1994; Hoyer, Hannon & Martin, 2002). Some receptors, including 5-HT_{1a}, 5-HT_{2a} and 5-HT_{2c} have been demonstrated to alter in sensitivity or number following exposure to treatment that increased serotonin release (Michelsen, Schmitz & Steinbusch, 2007) which is why these receptors have been the focus of PET studies when examining the effects of MDMA on serotonergic markers in human ecstasy users. In addition to their sensitivity to serotonin agonists, 5-HT_1, 5-HT_2 and 5-HT_4 are potentially involved in memory functions, since these receptors are present in regions that are crucial to cognition, such as the hippocampus and frontal cortex (Berumen, Rodriguez, Miledi & Garcia-Alocer (2012- see Figures 1.3-1.4). Furthermore, SERT plays a key role in serotonergic transmission and serotonin has been posited to support plasticity in the brain generally, and also supports long term potentiation in hippocampus, which is pivotal to the formation of enduring memories (Buhot, Martin & Segu, 2000; Michelsen, Prickaerts & Steinbusch, 2008).

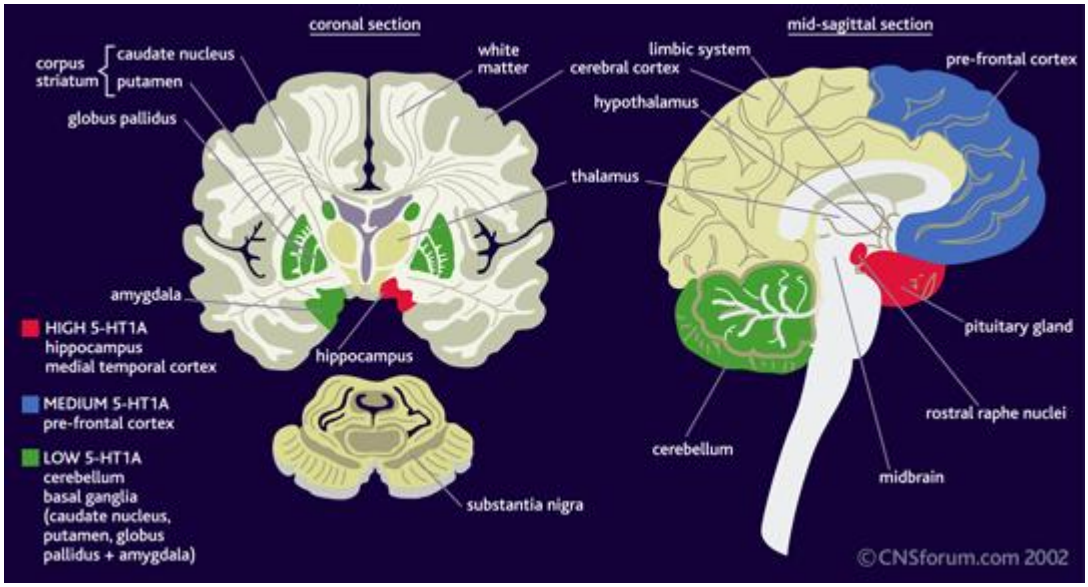

Figure 1.3 There are seven sub-types of 5-HT receptor and the 1A subtype is widely expressed throughout the brain. The highest levels of this sub-type are found in the hippocampus and medial temporal cortex, with slightly lower levels in the pre-frontal cortex. Low levels of 5-HT1A are found in the basal ganglia.

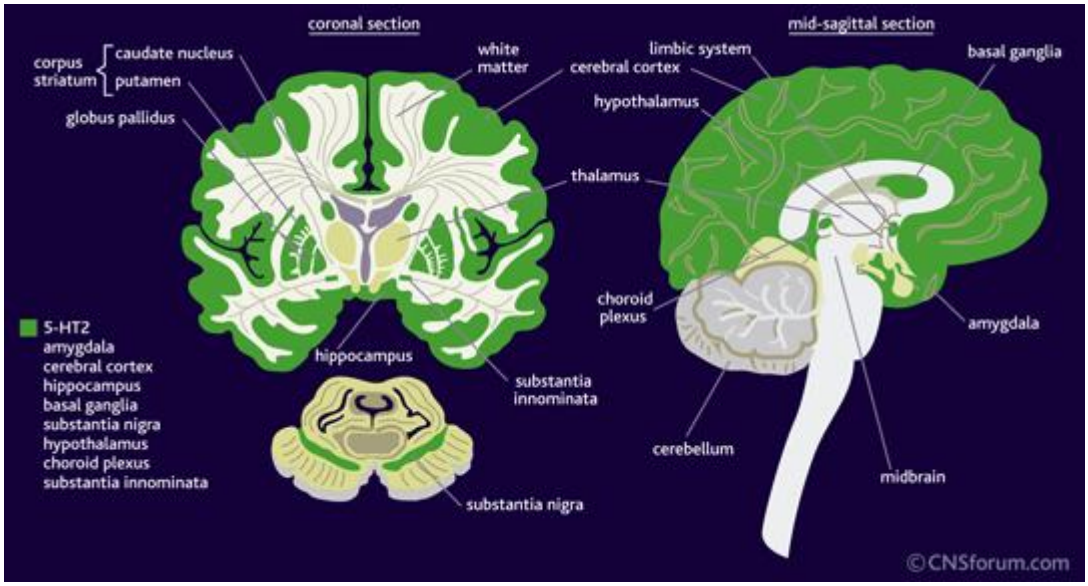

Figure 1.4. The 5-HT2 receptors (A, B and C subtypes) are widely distributed throughout the brain. These receptors can be found in the cerebral cortex, amygdala, hypothalamus, hippocampus, substantia nigra, choroid plexus, substantia innominata and some components of the basal ganglia. Images retrieved from: www.cnsforum.com/imagebank/section/Serotonergic/default.aspx

Studies on the long term effects of MDMA on serotonin in humans have used indirect measurement methods such as measurement of the serotonin metabolites, typically Positron Emission Tomography (PET) and Single Proton Emission Computed Tomography (SPECT). PET and SPECT require the administration of a compound that has been tagged with a radioactive isotope (referred to as a ligand) which allows the movement of the compound in the brain to be scanned and traced. The most common compound used in this research are [^{123}I]β-CIT, [^{11}C] McN5652 and [^{11}C]DASB. These techniques have been validated for the measurement of MDMA- induced serotonergic abnormalities in humans (Kish, 2002; Reneman et al., 2006). The PET radioligands (+)-[^{11}C]McN-5652 and [^{11}C]DASB have been found to selectively bind to serotonin transporters in the human brain (Frankle et al., 2004; Houle, Ginovart, Hussey, Meyer, & Wilson, 2000; Szabo et al., 1995). Other measures of the effects of MDMA in humans include Proton Magnetic Resonance Spectroscopy and the measurement of metabolites such as 5-Hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid of ecstasy users. Research using these techniques will be reviewed below, followed by a review of PET and SPECT studies, which comprise the bulk of the neuroimaging literature thus far.

Indirect measures of serotonergic alteration in ecstasy users; Cerbrospinal fluid measures and Proton MR Spectroscopy

As serotonin levels cannot be measured directly in the human brain, some early research measured the concentration of the major metabolite of serotonin (5-hydroxyindoleacetic acid; 5-HIAA) in the cerebrospinal fluid of ecstasy users as a substitute. One early study, with a small sample size of relatively novice ecstasy consumers (n = 5, lifetime dose ranging from 1-33 pills) reported no difference in 5-HIAA concentrations between ecstasy users and controls (Peroutka, 1987). In contrast, in the CSF of approximately 30 ecstasy users who had used ecstasy on more than 25 occasions and had been abstinent for two to three weeks, the metabolite concentrations of 5-HIAA were significantly lower compared with the levels of control participants (McCann, Mertl, Eligulashvili, & Ricaurte, 1999; McCann et al., 1994; Ricaurte, Finnegan, Irwin & Langston, 1990). There was no difference in levels of 4-hydroxy-3-methoxyphenylacetic acid, homovanillic acid (a metabolite of dopamine) between the two groups, suggesting that ecstasy

preferentially affects the serotonin system (McCann, Mertl, Eligulashvili, & Ricaurte, 1999; McCann et al., 1994). Only one study however, (Bolla, McCann, & Ricaurte, 1998) showed a significant negative correlation (r = -0.52, p<.013) between average monthly dose and 5-HIAA concentration.

Proton MR Spectroscopy (^{1}H-MRS) is used to detect various metabolites in the brain, including *N*-acetylaspartate (NAA) and myo-inositol (MI) both of which have been investigated in ecstasy users. A reduction of NAA represents a reliable non-specific marker for neuronal loss or dysfunction (Reneman et al., 2006) and an increase in MI may reflect a glial response to MDMA- induced axon degeneration (Cowan, 2007). Chang, Ernst, Grob and Poland (1999) reported increased MI in the right parietal lobe of MDMA users compared with controls, however Cowan et al., (2007) and Reneman, Majoie, Flick and den Heeten, (2002) failed to detect differences in MI in the occipital lobe and parietal lobe between ecstasy users and controls. For NAA, no differences were observed for the hippocampus bilaterally (Obergriesser, Ende, Braus & Henn 2001) or in the in the left hippocampus, mid-frontal grey matter or mid occipital grey matter of ecstasy users relative to controls (Daumann, Fischerman et al., 2004). Reneman, Schmand, and van den Heeten (2001) did report reduced NAA for ecstasy users in mid-frontal gray matter and also reported a significant correlation between impaired delayed recall scores on a word learning task and NAA concentration in the prefrontal cortex of ecstasy users. So far, H-MRS studies have lacked consistent results with regard to NAA and MI, although the sample sizes have been small (ranging from 5 to 21 for the ecstasy using group) and may have limited statistical power.

Voxel-based morphometry (VBM) is a neuroimaging technique that allows investigation of differences in brain anatomy. The volume of the whole brain or specific regions of interest are measured by drawing on images from MRI and calculating the volume. The output from this method is a statistical parametric map which shows differences in grey matter concentration between groups (Ashburner & Friston, 2000). Cowan et al. (2003) used VBM to compare overall regional brain grey matter density between ecstasy-poly-drug controls and drug naive participants. Lifetime ecstasy use ranged from five to over 40 occasions of use, and urine screens confirmed that participants were abstinent at the time of testing. Ecstasy users had

reduced grey-matter concentrations in various brain regions, particularly in the middle temporal gyrus, area V2 and bilateral parts of Broca's Area. Following up on reports of two cases of "acute hippocampal toxicity" following chronic ecstasy use, den Hollander et al. (2011) selectively investigated the hippocampal volume of long term ecstasy users compared with poly-drug using controls. Ecstasy users were male; had a minimum lifetime dose of 50 tablets and were required to be at least two weeks abstinent at the time of testing (confirmed by urine analysis). Hippocampal volume was measured by manually outlining the hippocampus in volumetric MRI scans, which showed that ecstasy user's hippocampal volume was, on average, 10.5% lower than poly-drug controls. VBM showed the proportion of overall grey-matter volume was on average, 4.6% lower in the ecstasy using group and there were no significant differences between total brain volume or left and right hippocampal volume. den Hollander et al. suggest that Cowan and colleagues did not find grey-matter variations in the hippocampus as VBM analyses are less powerful at detecting differences than region of interest analyses due to correction for multiple comparisons.

PET and SPECT neuroimaging studies investigating the effects of ecstasy on serotonin markers

Much of the PET and SPECT literature on the effects of ecstasy on the human brain has focussed on indirect markers of serotonergic integrity, such as the SERT (serotonin reuptake transporter). SERT is responsible for the reuptake of serotonin from the synaptic cleft to the presynaptic neuron and is therefore a potential marker for serotonergic axons. SERT is found along the entire axon, rather than at the synaptic terminal alone and SERT density is highest in the raphe nuclei and subcortical structures and also in the hippocampus and prefrontal cortex (Gurevich & Joyce, 1996).There is currently limited agreement with regard to the underlying mechanisms that reduce SERT binding; it may loss of serotonergic axons (Reneman, Booij, Majoie, Van Den Brink, & Den Heeten, 2001) or axonal loss associated with subsequent formations of new serotonin projections (Erritzoe et al., 2011) or a temporary down-regulation SERT to compensate for serotonin deficiency or excessive release (Kish, Fitzmaurice, Chang, Furukawa & Tong, 2010). The

location and magnitude of decreased SERT binding have been inconsistent in the human literature, which is briefly reviewed below.

Using PET in combination with a serotonin transporter ligand, several studies have reported lower SERT density for regular ecstasy users compared with controls, suggesting that MDMA use may induce neurotoxicity in humans (Buchert et al., 2003; De Win et al., 2004; 2008; McCann, Szabo, Scheffel, Dannals & Ricaurte, 1988; McCann et a., 2005; Reneman et al., 2001; Ricaurte, McCann, Szabo & Scheffel, 2000; Semple et al., 1999). In humans, some data indicates global SERT reductions (McCann et al,. 1988) some has revealed limited areas of SERT reduction (McCann et al., 2005; 2008) and yet others have reported reductions in striatal and cortical SERT (Buchert et al., 2007; Reneman et al., 2001) compared with no striatal or cortical SERT reductions (de Win et al., 2008; McCann et al., 2005). A few studies have shown that SERT binding was only reduced for heavy female ecstasy users and not males, suggesting that females may be more susceptible to MDMA induced functional impairments (Buchert et al., 2004; Reneman et al., 2001; Thomasius et al., 2003). Importantly, some studies were able to show an association between the degree of MDMA exposure and reduction in SERT levels (Buchert et al., 2003; McCann et al., 2005; Thomasius et al., 2003).

Two studies have compared markers for dopamine and serotonin functioning to investigate whether ecstasy selectively impairs serotonergic functioning. Semple et al. (1999) used SPECT and demonstrated reduced SERT density in the neocortex for ecstasy users (male only) compared with controls with no concurrent reduction in dopamine transporter density. The reduction in SERT density was positively correlated with abstinence from MDMA. McCann, Szabo, Vranesic et al. (2008) recruited participants who had taken two to three sequential doses of ecstasy within a short period of time (between 3 and 12 hours) in an attempt to mimic the high MDMA plasma concentrations found in research rats. High plasma concentrations in rats have been associated with dopaminergic as well as serotonergic alterations. McCann et al. used PET with DAT and SERT ligands to ascertain if there were any differential effects of ecstasy use on these neurotransmitters. The results showed a significant reduction in SERT binding for ecstasy users compared with controls in

the dorsolateral prefrontal cortex, orbitofrontal and parietal cortical areas. There were no differences in dopamine transporter binding between ecstasy users and controls.

Compared with SERT, less research has been conducted on 5-HT_{2a} receptors to date. 5-HT_{2a} receptors are distributed throughout the CNS, with dense concentrations in the neocortex(Capela, 2009). Located at the post-synapse of 5-HT neurons, they are therefore an indirect measure of synaptic serotonin release. The 5-HT_{2a} receptor has been shown to down-regulate with ongoing exposure to agonists and to up-regulate in their absence (Van Oekelen et al., 2003). Erritzoe et al. (2011) compared SERT and 5-HT_{2a} levels between people who had used both ecstasy and hallucinogens (hallucinogens being a class of drugs that also rely on serotonin release for their acute effects), but who had consumed one significantly more than the other. They reported significantly reduced SERT binding for the ecstasy preferring group (mean lifetime dose = 1296 tablets) in the amygdala, neocortex and pallidostriatum compared with the hallucinogen preferring group (mean lifetime ecstasy dose = 60 tablets). The reductions in SERT were correlated negatively with lifetime ecstasy use for both groups. 5-HT_{2a} receptor binding was slightly decreased for hallucinogen and ecstasy users relative to controls, although this effect was interpreted conservatively due to borderline statistical significance after removal of two potential outliers in the sample. Erritzoe and colleagues suggest that the lower binding of SERT may compensate for MDMA induced serotonin depletion, thus preventing post-synaptic receptors from up-regulating.

Deficits in connecting multiple brain regions

Recent research has suggested that the cognitive deficits associated with ecstasy use could be associated with compromised connections between different neural networks, possibly due to a reduction of serotonin in the thalamus. DeWin et al. (2008) used SPECT in combination the radioligand [^{123}I]b-carbomethoxy- 3b-(4-iodophenyl) tropane (CIT) and MRI to assess the functional and structural changes associated with ecstasy use. In this prospective cohort study, all participants were ecstasy naïve at baseline. At the 17 month follow up, 59 had taken Ecstasy (mean = 6 tablets) and the remaining 56 matched controls remained ecstasy naïve. After statistical adjustment for various other illicit substances, De Win et al., reported

decreased cerebral blood volume in the globus pallidus and putamen, and decreased fractional anisotropy (a measure of axonal integrity) and increased apparent diffusion coefficient in the thalamus. DeWin et al. suggest that these findings indicate ecstasy-induced damage to serotonergic axons caused by serotonin depletion. As the thalamus provides multiple connections between cortical and subcortical areas and is important in memory and language, DeWin et al. speculate that serotonergic alterations in this area may partly explain the memory deficits often reported for ecstasy users.

Due to the innervation of serotonin axons to the supplementary motor area, primary motor cortex and motor thalamus, and evidence suggesting that fluctuations in serotonin function may be associated with changes in functional connectivity within neural networks, recent research has examined the effect of ecstasy consumption on brain motor regions and the coordination of neural networks within this region (Karageorgiou et al., 2009, 2011). Using fMRI during performance of an event-related finger tapping task, Karageougiou (2009) reported no differences between ecstasy users and controls for finger tapping performance but greater event-related activation and amplitude for the ecstasy using group. Re-analysis of the data to assess functional connectivity revealed disruptions in thalamic communications within the thalamo-cortical-striatal behavioural control triangle for ecstasy users compared with controls (Karageougiou, 2011). Thus, lifetime ecstasy exposure was significantly, negatively correlated with diminished connectivity between seven region pairs within the motor system and amplitude and activation was higher during the finger tapping task. The authors suggest that ecstasy use resulted in increased thalamic activity and decreased functional connectivity. Put simply, ecstasy users motor regions worked with less collaboration and required more neural activity to perform at the same level as controls.

Serotonergic fluctuations may be transient

Early non-human studies on the effects of MDMA on the serotonin system indicated that although MDMA caused long-term changes, they could be reversible. Fischer, Hatzidimitriou, Wlos, Katz and Ricaurte (1995) raised serious concerns about the potential for MDMA to be neurotoxic to humans. They examined

serotonergic innervations patterns in rats and monkeys lesioned with MDMA in the previous 12-18 months. Fischer et al. reported that two weeks after MDMA exposure there was significant denervation of serotonin axons in various brain regions, more so for primates than rats. Seventy-two weeks post exposure, Fischer et al. observed that for most of the monkeys, there was re-innervation of axons, although the pattern of re-enervation was unusual, with areas distal to the raphe nuclei remaining denervated and areas more proximal to the raphe being re-innervated or hyper-enervated. Similalry, Hatzidimitriou, McCann and Ricaurte (1999) examined SERT densities in monkeys seven years after MDMA exposure and found long lasting reductions in SERT densities in the neo-cortex, hippocampus, cingulate cortex and amygdala, and recovered or hyper-enervation in the hypothalamus and globus pallidus respectively. There was no apparent loss of cell bodies in any brain region. These results suggested that although MDMA can cause structural alterations and denervation of serotonin axons, there is scope for regeneration in areas closer to the raphe nuclei. These patterns of serotonin axon alterations were consistent with Fischer et al. with regard to the distal and proximal nature of recovery.

In human studies that have compared former ecstasy users with current ecstasy users, there is additional evidence that serotonergic alterations as a consequence of MDMA exposure may be transient. For example, Buchert et al. (2003, 2004) and Thomasius et al. (2003, 2006) recruited poly-drug users, current ecstasy users and former ecstasy users (abstinent for at least 5 months as confirmed by hair analysis) and compared SERT densities between groups. PET scans revealed significant reductions in SERT density in the mesencephalon of current users compared with the other groups, and reduced SERT density in the caudate nucleus compared to the poly-drug group only. The mesencephalon effects were best predicted by number of tablets taken within the preceding year (R_2=0.12, p <.001) and number of occasions of ecstasy use was the best predictor for effects in the caudate nucleus (R_2=0.07, p = 0.004). There were no significant differences between former ecstasy users and poly-drug users in SERT densities (Thomasius et al., 2003). Buchert et al. (2004) reported significant reduction in SERT density for current users compared with drug naive, poly drug and former ecstasy users in multiple brain areas, including the mesencephalon, thalamus, occipital cortex and hippocampus.

Consistent with Thomasius et al., (2003) there were no significant differences between poly-drug and drug naïve groups, suggesting these SERT effects may be reversible. There was also a significant correlation between SERT density and period of abstinence in the basal forebrain and brainstem regions of current ecstasy users. Using PET and the SERT ligand [^{11}C]DASB, Selvaraj et al. (2009) recruited former male ecstasy users and former male poly-drug users who had been abstinent for one year, in an attempt to ascertain how long SERT reductions are present as a consequence of ecstasy use. There were no differences in SERT binding between former ecstasy users, former poly-drug users and drug naive controls and no significant correlations between pattern of ecstasy use and SERT binding. Selvaraj et al. suggest that a period of prolonged abstinence from ecstasy allows recovery of SERT availability. These studies suggest that MDMA may alter serotonin availability in the CNS but this alteration may be reversible after a period of abstinence from ecstasy.

Kish et al (2010) used PET with the SERT ligand [^{11}C]DASB and MRI with voxel-based analyses to assess SERT density in ecstasy users and controls. They reported no differences between ecstasy users (who typically used one to two tablets per session) and polydrug controls in various SERT dense areas, such as the thalamus and striatum, however SERT binding was significantly lower in the cerebral cortex (particularly the hippocampus and occipital cortex). The ecstasy group reported lower subjective mood relative to controls, and showed "modest" deficits on tests of executive function, attention and memory, the latter being associated with SERT decrements in the hippocampus. Importantly, the reduction in SERT binding was not related to structural changes or partial volume effect (which addressed potential bias arising from possible reduced brain volume in the ecstasy group) recent use of other stimulant drugs (confirmed by hair analysis) blood testosterone or oestradiol levels, major SERT gene promoter polymorphisms, gender, psychiatric status, or self-reported hyperthermia or tolerance. Consistent with previous research (eg. Thomasius et al., 2003; Buchert et al., 2005, McCann et al., 2005) Kish et al. also reported that maximum single dose of ecstasy taken (F (2,44) = 9.57, p <.001) and number of years of ecstasy use (F (1,41) = 4.91, p =

0.032) were the best predictors of SERT binding in all cortical areas and also in the caudate nucleus, respectively.

Three case studies of ecstasy users have recently demonstrated ecstasy induced neurotoxicity to the human brain. The first two isolated cases followed ingestion of ecstasy (1-2 tablets) which resulted in the persons experiencing seizures. Forty-eight hours post seizure, MRI showed significant swelling and altered signal volume in the right hippocampus in both users (one male, one female). Three months post seizure, both ecstasy consumers denied having taken subsequent doses since the seizures and both had ongoing right hippocampal atrophy and ongoing high signal volume in the hippocampus. Although the authors could not rule out an infectious encephalitic cause or continued subclinical seizure activity, they suggested that in these cases, ecstasy ingestion caused an "acute toxic insult to the hippocampus" leading to MRI changes and seizures (Gardner, Lawn, Fatovich & Archer, 2009). In addition to these cases, the first direct measurement of serotonin markers in a human (post mortem) brain of a heavy ecstasy user was reported on by Kish, Fitzmaurice, Chang, Furukawa and Tong (2010). The authors reported decreased levels of serotonin, as well as the related markers of 5-HIAA, protein concentrations of tryptophan hydroxylase and SERT, thus providing more direct evidence that serotonergic neurotransmission is altered by ecstasy use in humans.

Overall, there appears to be adequate evidence that ecstasy use is associated with alterations to the human serotonergic system. Serotonin is associated with smooth synaptic transmission and is involved in long term potentiation in the hippocampus and serotonergic axons project to cortical and subcortical brain areas associated with cognition, including the frontal cortex and hippocampus, and both these regions also contain serotonin receptors 5-HT_1 and 5-HT_2 (Michelsen, Prickaerts & Steinbusch, 2008). Reduced SERT densities in the prefrontal cortex, occipital lobe, thalamus, hippocampus and in cortical areas generally have been reported for ecstasy users relative to non-users (Erritzoe et al., 2011; Kish, Fitzmaurice, Chang, Furukawa & Tong, 2010; Reneman, Booij, Majoie, Van Den Brink, & Den Heeten, 2001) and this ecstasy related effect is specific to SERT rather than DAT, suggesting specific, longer term alterations to serotonergic rather than

dopaminergic transmission with regular ecstasy use (McCann et al., 2008). These effects may be temporary however, with lower SERT density reported for current users compare with former users (Buchert et al., 2005; Selvaraj et al., 2009). Reduced SERT density has been demonstrated to correlate with poor memory performance and measures of previous ecstasy exposure (Thomasius et al., 2003; Buchert et al., 2005, McCann et al., 2005) and emerging prospective cohort, neuroimaging studies indicate that even after relatively few lifetime occasions of use, axonal injury can occur (De Win et al., 2008).

Chapter 4.

Components of Memory and Measures of Verbal Memory Relevant to the Present Thesis.

The previous chapter reviewed the evidence for serotonergic irregularities associated with recreational ecstasy use, and found that these irregularities were sometimes correlated with poor memory performance. Verbal memory deficits have frequently been reported or regular ecstasy users (reviewed in detail in Chapter 6) and as the present thesis investigates components of verbal memory and learning that may be vulnerable to the effects of regular ecstasy use, this chapter reviews some of the cognitive processes inherent to verbal memory performance, and the ways in which these processes have been assessed using tests from neuropsychological practice.

Learning is the process of acquiring new information which has the potential to alter behaviour. Memory includes the acquisition of new information and its subsequent storage and retrieval, as such it is intricately involved with learning (Lezak, Howieson, & Loring, 2004; Purdy, Markham, Schwartz, & Gordon, 2001). Memory may therefore be defined as a lasting, internal representation of a past event or experience (or an aspect of it) that is reflected in thought or behaviour (Moscovitch, 2007, p. 17). By analysing memory as a collection of heterogeneous hypothetical constructs, cognitive psychology has made significant contributions to our understanding of memorial processes. Although this fractionation of memory has led to some criticism that cognitive psychology relies too heavily on un-tested concepts and models, insights from research participants with brain lesions and from neuroimaging studies have begun to link these concepts with specific memory processes and brain regions, and these neural correlates have been incorporated into memory models (Moscovitch, 1992; Tranel & Damasio, 2002).

Short term memory (also referred to as immediate or primary memory) is of short duration (about one minute) and has limited capacity. Short term memory is often tested by span tasks, such as Digit Span Forwards. This test involves the verbal presentation of a series of numbers, increasing in length from two to nine digits, and

requires the participant to repeat them back in the same order of presentation. Word span tasks are similar, and consist of the presentation of increasing strings of one syllable words. The number of digits or words recalled in the correct order is a measure of short term memory capacity. How much information can be stored in short term memory is debatable, although George Miller's famous paper, *The Magic Number 7±2* (Miller, 1956) indicated that between 5 and 9 items can be simultaneously held in STM, a range that is still reported in short term memory scores (Salmon & Squire, 2009). Strategies such as chunking and rehearsal can be used to maximise short term memory performance (Purdy, Markham, Schwartz & Gordon, 2001). Short term memory is heavily dependent on the allocation of attention, if attention is directed elsewhere during span tasks, items in short term memory are likely to be lost (Woodard, 2006).

Short term memory is used during recall of immediately presented information that is no longer physically present, and passively stores information for a limited time. This is in contrast to *working memory*, which refers to the ability to store a limited amount of information and also to manipulate that information (Baddely & Hitch, 1974; 1994). Working memory was termed by Atkinson and Shiffrin (1968) who suggested that information could be stored in working memory so other cognitive operations, such as re-ordering, could be performed on it. Working memory is often tested using the Digit Span Backwards task, which is identical to the Digit Span Forwards task except that the participant is instructed to recall the digit string in the reverse order to which it was presented, thus requiring mental manipulation of the numbers. The prevailing model of working memory has been Baddely and Hitch's,(1974) who have conceptualised working memory as an interaction between verbal short term memory (termed the phonological loop in order to express the sub vocal rehearsal required to prevent decay of information) the visiospatial sketchpad (responsible for storage and manipulation of non-verbal information), and the central executive (posited to selectively attend to certain task demands while suppressing irrelevant information, and regulating the operations of the short term systems). Later the episodic buffer was added to the model, which is a limited capacity store that binds or links information across different areas to form integrated episodes. This slave component is also under the intentional and

conscious control of the central executive (Baddeley, 2000). The premise of a system dedicated to cognitive control has been widely accepted in theories of cognition, and may include processes such as attentional control (Shallice, 1986) self-regulatory control (Barkley, 1997) updating, inhibition, and shifting (Miyake et al., 2000) selective attention, decision making, response inhibition (Miller & Cohen, 2001) and planning and strategising goal directed behavior (Lezak, 2004). All of these skills are termed *executive functions* and results from cognitive studies of persons with frontal lobe lesions and neuroimaging research has identified them as being dependent on the integrity of the prefrontal cortex (eg. Alvaroy & Emory, 2006; Baldo & Shimamura, 2003; Lezak, 2004).

Well-researched theories of memory have identified two types of *long term memory*, declarative (also referred to as explicit) memory and non-declarative (or implicit) memory. Declarative memory is the conscious and deliberate recollection of facts and events that can be "declared" and includes memory for the words on a recently presented list (also an example of episodic memory: memory for events) and knowledge that a dog is an animal (this is an example of semantic memory: memory for facts and meaning of words). This is in contrast to implicit, non-declarative memory because an implicit memory cannot be consciously recalled and stated. Implicit memory arises from priming, when participants perform better on tasks for which they have been unconsciously prepared for. Presenting a study list of words, followed by a word fragment completion task in which the study words appear with missing letters is an example of an implicit priming task. Procedural memory (memory for a series of actions, such as actions required to drive a car) is an example of implicit memory (Cohen & Squire, 1980; Tulving & Madigan, 1970; Tulving, 1983; Otto & Eichenbaum, 1992). Declarative memory requires the storage and recollection of temporally dated events that depend upon temporal, or spatial contextual cues for accurate retrieval. Although it is not possible to map a memory task to a memory process on a one-to-one basis recalling a list of words presented 20 minutes earlier is widely regarded as a test of verbal declarative memory (Tulving, 2002). Source memory, which refers to the recollection of the context in which an item was encountered (eg. being able to recall whether a previously presented word was in written or spoken form) is also a type of episodic memory (Baddely,

Kopelman & Wilson, 2002; Salmon & Squire, 2009). The focus of the current research is on verbal declarative memory, which is defined as the conscious and deliberate recollection of words that were previously learnt or that participants have consciously tried to learn.

Most memory theories are to some extent, mediationist; they adhere to the idea that to form a memory, three processes take place; acquisition (which is dependent on encoding and consolidation), storage and retrieval (Watkins, 1990). *Encoding* refers to the accurate perception of information, which is transformed into a perceptual or conceptual format so that it can be stored into long term memory. Encoding is thus the processing of information to be stored. The process of encoding causes a change in the central nervous system, such that the perception of a stimulus leads to a corresponding *mental representation*. This term is sometimes used interchangeably with *memory trace*, or *engram*, all of which are hypothetical constructs to describe how a representation of external stimuli is created in the brain. Thus, encoding is the process of transforming an external stimulus into a mental representation. This initial perception includes properties of the individual item, the familiarity of the item, and its relationship to the context in which it was presented.

Encoding is the first process of *learning*, which Lezak (2004) defines as the *acquisition* of new information. Whether or not a person can learn something depends partly on how often the stimuli are repeated or rehearsed. Repetition of stimuli can strengthen the duration of a memory trace, thus fortifying encoding and speeding up the consolidation process. Repeated presentations of a word list for example, allow more items to be learnt, and thus participants can expand recall beyond short term memory span (Tulving & Madigan, 1970). With repeated exposure to stimuli, the signalling strength of neural circuits involved in processing is reinforced, ultimately becoming more automated and "hardwired." This process is referred to as long term potentiation, and is believed to be the cellular mechanism underlying learning and memory (Bliss & Collingridge, 1993; Tranel & Damasio, 2002). The hippocampus is well positioned to host long term potentiation due to its high interconnectivity with the cerebral cortex and dense serotonin receptor sites (Molodstova, 2008). The hippocampus is also believed to support c*onsolidation*,

which refers to the memory trace becoming stronger after encoding, consequently becoming more durable over time (Squire, Stark & Clark, 2004; Wixted, 2005). Some theories postulate that consolidation can be disrupted, and hence recall impaired, when there has been a high degree of mental exertion during encoding (Wixted, 2004). *Storage*, sometimes referred to as *retention*, occurs when the memory trace becomes a permanent, stable record of information in the brain (Tulving & Madigan, 1970; Tulving & Thomson, 1973; Purdy et al., 2001). Storage is thus the consequence of the process of consolidation. Although the memory trace is consolidated in the medial temporal lobe, long term declarative memories are ultimately distributed throughout the cerebral cortex. This theory accounts for why new learning cannot take place when the hippocampus is severely damaged but previously stored information can be retrieved (Gabrieli, 1998; Squie & Zola-Morgan, 1991; Squire et al., 2004). In list learning tasks, the traditional measure of storage/retention is delayed Recognition performance, as this measure provides a word as a memory cue, thus eliminating the need for a retrieval search.

Retrieval of information is an interactive process that involves search processes that identify the correct context in which an item was learnt (eg. recalling a time when a list of words was learnt) and from there, searching for the correct items and copying information from long term memory into short term memory. Evidence from Craik and Lockhart's (1972) famous levels of processing paradigm, as well as evidence from list learning tasks that present categorical to-be-remembered items (Gershberg & Shimamura, 1995) indicate that the more an item can be related to pre-existing knowledge or organised with others at encoding, the greater the likelihood of retrieval. Retrieval of information can be achieved via free recall, cued recall or recognition. In list learning tasks, retrieval of presumably consolidated words is assessed by free recall after a delay. Retrieval demands greater cognitive processing as it requires temporarily copying stored information into consciousness, via short term or working memory, to be utilised for the current task demands (Watkins, 1990). For example, free recall requires the retrieval of words from a list that was learnt 30 minutes previously. Recognition, on the other hand, only requires that one decides whether one of several items was encountered previously. Unlike recognition, free recall does not provide any cues, and as such is a more difficult task

(Tulving & Madigan, 1970). Most memory researchers accepts the idea that recognition consists of dual processes and currently the most prominent theory distinguishes between recollection and familiarity (Curran & Hintzman, 1995; Yonelinas, 1998). Recollection is episodic as it includes information about the episode and context in which an item was encountered, whereas familiarity is a "knowing" that an item was encountered in the absence of contextual information. The theory assumes that stronger memories are subsumed by recollection, but when recollection fails, weaker memory relies on familiarity (Eichenbaum, Yonelinas & Ranganath, 2007). Evidence from fMRI data suggests that recollection is the function of the hippocampus, and familiarity is mediated by the adjacent areas of the medial temporal lobe (MTL, Brown & Aggleton, 2001; Eichenbaum et al., 2007; Rugg & Yonelinas, 2003) although Wais (2008) performed a meta-analysis on the recognition-based fMRI research and argued that fMRI evidence indicates there are different regions associated with strong (hippocampus) and weak (parahippocampal cortex) memory strengths.

Neuroimaging memory processes

The mediationist account of memorial processes has been influential in the development of neuropsychological tests that provide information on processes that are susceptible to neurological conditions, and this has resulted in a large catalogue of research comparing measures of list learning between persons with localised brain injuries. These studies have provided a wealth of data on how damage to certain regions impair memory scores, however a problem in the interpretation of both neuropsychological data and lesion studies is the inability to differentiate between the processing stages of memory. For example, hippocampal lesions impair free recall of newly learnt material, however it is not possible to determine whether this arises from a deficit in encoding or a deficit in retrieval. Further, lesion studies potentially confound the effects of localised injury and disruption to a region with alterations in neurotransmitter regulation as a consequence of injury. Functional magnetic resonance imaging is able to address these shortcomings, particularly with the advent of event related fMRI (efMRI) which shows the neural activity associated with a specific stimulus (as opposed to previous block designs which were an average of activity over repeated stimuli presentations). This means that events (eg.

neural response to a word) can be categorised post hoc on the basis of a participant's behaviour, such as whether the word was subsequently recalled correctly or forgotten. This subsequent memory paradigm is used in conjunction with efMRI to identify the neural correlates of encoding. This paradigm consists of a study phase (during which imaging takes place while a participant intentionally tries to encode words) and a recognition phase, when old items are interspersed with new items. The activity that is elicited by study items can be correlated with whether they were subsequently judged correctly as old. Such subsequent memory effects link encoding related-neural activity with a behavioural measure of memory (i.e remembering or forgetting). The neural correlates of retrieval can be assessed by imaging during the recognition phase, with activity associated with correct judgments being assumed to underlie retrieval regions (Henson, 2005). An overview of memory processes involved in verbal list learning and their neural correlates is presented below.

Medial temporal lobe

An extensive literature has identified the medial temporal lobes (MTL) as being critical for the encoding of verbal, episodic memories. Within the MTL, the hippocampus (including the dentate gyrus), the entorhinal and perirhinal cortices, and the parahippocampal gyrus are typically identified as key structures for encoding and consolidating verbal items. The benchmark finding from studies of MTL lesions in humans is profound forgetting of recently presented verbal and visual information when immediate memory is intact (for reviews see: Aggleton & Brown, 2006; Eichenbaum, 2000; Gray & McNaughton, 1983; Manns & Squire, 2002; McClelland, McNaughton, O'Reilly & Randall, 1995; Squire & Stuart, 1991; Squire, 1992; Squire, Stark & Clark, 2004; Winocur & Moscovitch, 2011). Even for people with large MTL lesions, digit span is normal (Cave & Squire, 1992; Gagnon, Foster, Turcotte & Jongenelis, 2004).

Early neuroimaging experiments of hippocampal activation during verbal memory tasks did not corroborate the lesion studies to the extent that was anticipated, however more recent meta-analyses have begun to consistently report activation of the hippocampus across studies (Henson, 2005). Studies have reported subsequent memory effects during verbal tasks for the hippocampus during encoding

(Cabeza & Nyberg, 1997; Daselaar, Veltman, Rombouts, Raaijmakers & Jonker, 2003; Fletcher, Stephenson, Carpenter, Donovan, & Bullmore, 2003; Morcom, Good, Frackowiak, & Rugg, 2003; Otten, Henson, & Rugg, 2001) and during successful retrieval of verbal items (Cabeza, Dolcos, Graham & Nyberg, 2002; Cohn, Moscovitch, Lahat & McAndrews, 2009; Greicius et al., 2003; Jonides, Wager & Badre, 2002). Lapage, Habib and Tulving (1998) conducted a review of 52 PET studies of episodic memory and reported changes in BOLD response associated with encoding and retrieval in the rostral and caudal portions of the hippocampus respectively. By designating sites as caudal or rostral based on their proximity to predetermined markers, the authors summarised that 83% of rostral sites were activated during encoding, and 94% of the caudal sites were occupied by the retrieval conditions. In a meta-analysis of 17 studies investigating the neural correlates of the dual process theories of recognition memory, Wais (2008) reported that the hippocampus was involved in recollection and the perihinal cortex subserved familiarity. Wais reinterpreted these results based on an alternative dual process model which posits that when the hippocampus is engaged, strong memory results and when the perihinal cortex is activated, weaker memory results. In an attempt to quantify the existing efMRI studies of episodic encoding and recollection, Spaniol et al. (2009) conducted a meta-analysis to identify the significant concordances in brain activity patterns across independent studies using the Activation Likelihood Estimate (ALE) method. The meta-analysis consisted of 26 efMRI studies that used subsequent memory paradigms for either encoding or retrieval success. The meta-analysis showed that activation of the MTL was reliably evident in efMRI studies of memory. Within the MTL, the left anterior hippocampus and right amygdala were more strongly associated with encoding than retrieval, and the left parahippocampal gyrus was more strongly activated during retrieval. A recent ALE based meta-analysis which limited inclusion to studies that conducted whole brain analyses during encoding of visually presented words reported subsequent memory effects for the left anterior hippocampus (Kim, 2010). The findings from these later meta-analyses indicated preferential involvement of anterior hippocampus during encoding and as such are consistent with the results from the early review of Lapage et al. (1998). Overall, the evidence indicates that the MTL and particularly the

hippocampus support the encoding and recall of previously experienced events, such as lists of words.

Frontal cortex

Although the importance of the medial temporal lobes to memory has been recognised for at least fifty years, the contribution of the frontal lobes has only been acknowledged in the last decade. Several neuroimaging studies have shown that the level of activity in the prefrontal cortex can predict whether an item will be subsequently recalled or forgotten. Early PET studies using verbal memory tasks reported subsequent memory effects for the left prefrontal cortex, activation of which was greater when participants engaged in semantic encoding strategies, such as deciding if a word is living or non-living (Kapur et al., 1994; 1996). Several fMRI studies later replicated these results, showing that prefrontal activation during encoding could predict accurate retrieval (Baker, Sanders, Maccotta & Buckner, 2001; Buckner, Kelley & Petersen, 1999; Kirchoff & Wagner, 2000) and that greater activation was associated with semantic judgements about words compared with structural judgements (Daselaar, Veltman, Rombouts, Raaijmakers & Jonker, 2003; Demb et al., 1995; Wagner et al., 1998). In a review of thirty-seven studies that investigated the frontal neural correlates of long-term memory encoding, Blumenfeld and Ranganath (2007) identified that activation in the ventrolateral prefrontal cortex (VLPC) was associated with subsequent recollection of items that had been encoded under semantic processing instructions. Activation in the dorsolateral prefrontal cortex (DLPC) was more likely to occur during encoding where some form of organisation or manipulation of items had to occur, such as chunking or serial reordering. The authors concluded that the VLPC may contribute to long term memory by selecting relevant item information at encoding, whereas the DLPC may support long term memory during encoding by building associations between items. Further evidence for the role of VLPC and DLPC in episodic memory encoding came from Spaniol et al's. (2009) meta-analysis, who reported similar activations of these regions across 26 efMRI studies of subsequent memory effects and activation of the left inferior frontal cortex and left anterior fusiform cortex were evident across 31 fMRI studies involving encoding of visually presented words (Kim, 2010). It has been suggested that the prefrontal cortex contributes to verbal episodic memory by

selecting task relevant information to assist in encoding, such as implementing strategies to find associations between items to assist in encoding (Blumenfeld & Ranganath, 2008; Moscovitch, 1992; Shimamura, 2000) and to search for cues and implement strategies to assist with retrieval (Buckner et al., 1999; Rugg & Wilding, 2000).

The utility of eFMRI has been extensive in the memory literature however a brief consideration of the caveats to this method is warranted. Firstly, any kind of retrieval paradigm is not "process pure" (because retrieval is always preceded by encoding and consolidation, and recognition itself relies on multiple processes). Secondly, efMRI is not suited to study the neural correlates of free recall, as there is no clear interval or stimulus to lock imaging to, resulting in too much noise. Most noteworthy though, neuroimaging data is ultimately correlational, it can provide information on which regional activations are associated with subsequent memory effects, but it cannot identify whether or not this activation is *necessary* for memory processing. For this, lesion studies are more effective as they provide information on how damage to a specific region disrupts memory (Logothetis, 2008). Nevertheless, efMRI is currently the most effective means of examining memorial processes with neuroimaging (Rugg, 2002) and neuroimaging studies have revealed a number of neuronal circuits that are associated with memory processes, and recent meta-analyses of this data have demonstrated consistent roles for the prefrontal and medial temporal structures in encoding and retrieval.

To summarise, prefrontal areas, including the ventrolateral prefrontal cortex and dorsolateral prefrontal cortex contribute to the selection of task relevant information and semantic properties of verbal items to assist with encoding and consolidation in the hippocampus, and during retrieval the prefrontal cortex plays a role in memory search and selecting retrieval cues and strategies. The MTL and frontal cortex appear to be involved in transforming verbal stimuli into a mental representation, which can later be retrieved. Since episodic memory involves both the MTL and prefrontal cortex, several researchers have proposed that the formation of memory relies on an interaction between these regions (eg. Becker & Lim, 2003; Blumenfeld & Ranganath, 2008; Kelley et al., 1998; Kirchhoff, Wagner, Maril &

Stern, 2000; Moscovitch, 1992; Moscovitch & Winocur, 2002; Shimamura, 2000; Simons & Spiers, 2003; Tranel & Demasio, 2002). Moscovitch and Winocur (1992) used the term *working with memory* to describe the relative contributions of the frontal and medial-temporal systems to encode, store and retrieve memories.

The Working with Memory Model

In contrast to the purely cognitive models of memory, the *Working with Memory* model has a strong neuropsychological basis and its formulations were arrived at from integrating the neuropsychological literature from perception, attention, memory as well as lesion and fMRI studies of memory. Many of the model's propositions, such as the idea that encoding and retrieval of memories depend on the interaction between prefrontal and MTL regions, have been become standard in the memory research field.

The *Working with Memory* model has four components; the posterior neocortex, which mediates performance on implicit memory tests, the parietal cortex, which is responsible for directing attention to stimuli, the medial temporal lobes, which automatically stores information and mandatorily recovers information that is cue driven, and the frontal lobes, which work with the MTL by supporting strategic processes that are required for encoding and retrieval (Figure 4.1). When information is consciously apprehended, the hippocampal component mandatorily takes on that information and binds those neural elements from the neo-cortex that mediates the conscious experience of an event. The hippocampal component is therefore required to bind together information from the neo-cortex to maintain cohesion between the various neural activations that represent an event. This process is assumed to be subserved by reciprocal pathways connecting the hippocampus and neo-cortex via the entorhinal cortex. The episodic memory trace is thus a cohesion of neocortical and hippocampal neurons. The hippocampal contingent is a sparse neural network that represents the trace. This acts as an index which activates the neo-cortical neurons comprising the content and the conscious experience of an event. Retrieval occurs when an internal or external cue triggers the hippocampal trace (index) which activates the neo-cortical ensemble bound with it. Retrieving episodic memories therefore entails retrieving the content and conscious experience of the

event. This recovered consciousness is akin to recollection- which, as mentioned previously, has been demonstrated to be a process mediated by the hippocampus. According to the Moscovitch model, recollection is a two staged process. The first stage is an automatic, rapid process in which retrieval may be unconscious, as is the case for procedural memory. The second, slower stage involves the conscious apprehension of the memory trace, which is then used to direct further action, such as searching for other items associated with the memory trace.

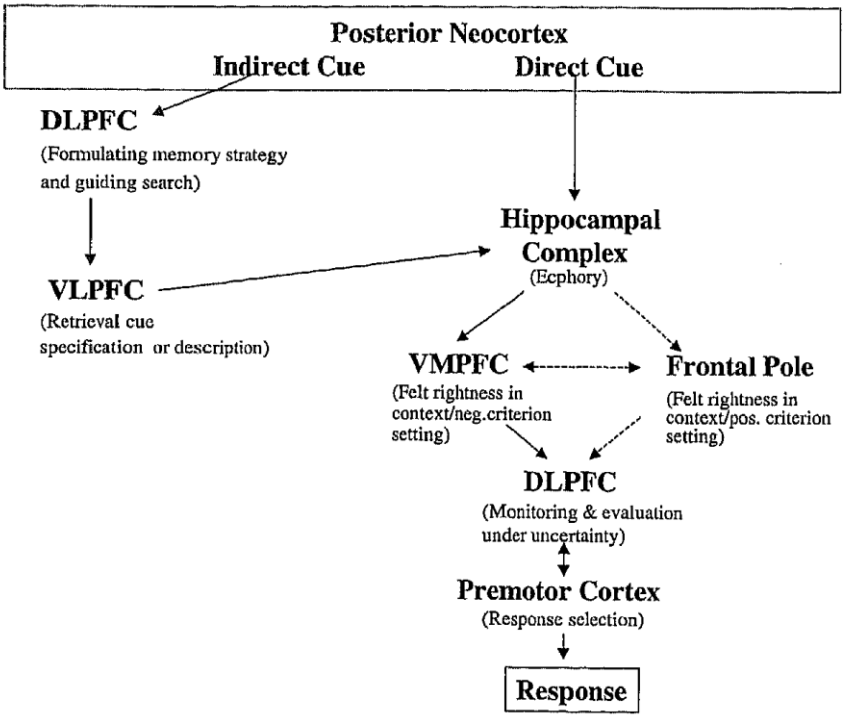

Figure 4.1. Flow diagram for interactions among medial temporal cortex and regions of the frontal cortex during retrieval of episodic memories. When presented with an indirect cue (such as recalling words from a previously encountered word list) the DLPFC supports retrieval strategies and the VLPFC fine specifies a cue. The frontal components work with the hippocampal complex to access recently consolidated items. When the cue is direct (such as in recognition tasks) the strategic frontal component may not be required, as there is an automatic interaction between the cue and the MTL- referred to here as ecphory. Image from Moscovitch and Winocur (2002, p. 204).

The hippocampal component is "stupid" in that it encodes without discernment, and it encodes at a shallow level. Memories have no semantic meaning

or organisational structure, they are simply stored "*like beads in a jar*" (Moscovitch, 2008, *p*. 65) in the MTL and throughout the neo-cortex. The reason for this is that humans cannot anticipate when a particular piece of information will be required, so all is encoded, and what is retrieved is retrieved either automatically, in response to a cue (as in the case with tests of recognition) or deliberately, as a consequence of an internally generated cue- a process that is mediated by the frontal component. Thus, the frontal component works with the hippocampal component, it controls the information delivered to the MTL, initiates and guides retrieval and helps to interpret and organise the information to be encoded and retrieved. Selecting contextual information at encoding, and cue specification and memory search processes at retrieval have all been attributed to the pre-frontal cortex by various authors (eg. Blumenfeld & Ranganath, 2007; Shallice, 2002; Simons & Spiers, 2003). The frontal component is needed to "confer intelligence" on the hippocampal system, it is required "*to string the hippocampal beads into different necklaces to be worn as befits the occasion*" (Moscovitch, 2008, *p*. 65).

The hippocampal component includes the parahippocampal gyrus, the entorhinal and perihinal cortices. Amnesia in humans is associated with bilateral damage to any one of those structures, and they have been shown to be activated in fMRI studies of encoding and retrieval. Memory impairments associated with damage to the frontal lobes also exist, however these tend be associated with impaired strategy selection for encoding and organisation rather than a memory deficit per se (eg. Alexander, Stuss & Gillingham, 2008; Fletcher & Henson, 2001; Shimamura, Janowsky & Squire, 1990). As there are multiple, reciprocal pathways between the hippocampus and frontal cortex, and both regions are innervated by serotonergic neurons (Barnes & Sharp, 1999; Hornung, 2003; Hoyer et al., 1994; Michelsen, Schmitz & Steinbusch, 2007) the hippocampal and frontal components of this model provide a useful framework for understanding why deficits in verbal learning might occur in ecstasy users.

Chapter 5
Assessing Verbal Memory Using List Learning Tasks

Verbal declarative memory requires adequate encoding, consolidation and retrieval and these processes can be distinguished on standard tests of verbal learning. List learning tasks have a long history in neuropsychology and have become an invaluable tool for evaluating memory functioning. Since learning a list of items which later need to be recalled is a relatively common occurrence, list learning tasks also provide a degree of ecological validity. List learning tasks have also been developed in accordance with concepts from cognitive psychology, and attempt to assess the processes underlying verbal learning and memory, such as immediate memory span, rate of acquisition, long term memory storage and retrieval and encoding strategies.

The Rey Auditory Verbal Learning Task (AVLT, Rey, 1958) is one of the most widely used tests both in clinical practice and research, and has been shown to effectively detect verbal memory deficits in a range of populations (Bigler, Rosa, Shultz, Hall, & Harris, 1989; Geffen, Butterworth, Forrester, & Geffen, 1994; Guilmette & Rasile, 1995; Janowsky, Shimamura, Kirtchevsky, & Squire, 1989; Lucas & Sonnenberg, 1996; Schmidt, 1996; Woodard, Dunlosky & Salthouse, 1999; Woodard, 2006). The AVLT has also been a very popular test of verbal memory in the ecstasy and memory research to date. The California Verbal Learning Test (CVLT, Delis Kramer, Kaplan & Ober, 1987) is also a widely administered test that has been shown to differentiate between healthy controls and persons with various neurological conditions (Baldo, Delis, Kramer, & Shimamura, 2002; Delis et al., 2005; Donders & Nienhuis, 2007).

The AVLT and CVLT are both multi-trial list learning tasks. They involve five presentations of a list of 16 semantically related (CVLT) or 15 unrelated (AVLT) words, which are read aloud to participants at a rate of one word per second. After each presentation, participants are asked to recall as many words as they can remember, in any order (free recall). This procedure is repeated five times, and allows an evaluation of the number of words recalled over the five trials. Delayed

recall is assessed after a 20-30 minute delay, after which a forced choice (yes or no) recognition test is administered, which consists of a longer list of words, half of which are from the original list and the remainder being distracters.

The primary difference between the AVLT and the CVLT is that the latter consists of semantically related items. Words from the same category are never presented consecutively in the CVLT, allowing examinees to organise the verbal material in such a way that can maximise recall. Two forms of organisation are serial-order clustering and semantic clustering. Clustering words serially involves organising them on the basis of presentation order, which is a shallow level of processing. Serial order clustering also elicits primacy and recency effects, which is the tendency to recall the words at the beginning and end of the word list, at the expense of words from the middle of the list. Semantic clustering involves organising words based on simular semantic features. For the CVLT, this involves grouping items according to one of four categories (eg. fruits, spices, tools) which imposes greater meaning on to-be –remembered words and allows for a deeper level of processing and better long term retention (Craik & Lockhart, 1972; Delis et al., 1987). Participants with lesions in the prefrontal cortex have been reported to have semantic clustering deficits on semantically related lists (eg. Alexander, Stuss & Fansabedian, 2003; Gershberg & Shimamura, 1995; Raz, 2000), frontal lesions impair semantic clustering more than serial clustering (Mangeles, 1997) and higher density of frontal lobe and not hippocampal volume has been shown to be associated higher semantic clustering on the CVLT (Kramer et al., 2005).

Measures derived from list learning tasks used in ecstasy research

The AVLT and the CVLT are both considered to be test of *verbal learning and memory* (Spreen & Strauss, 1998) and these terms are sometimes used interchangeably within the ecstasy and memory literature (eg. Indlekofer et al., 2009; Kalechstein et al., 2007; Zakzanis ety al., 2007). Care needs to be taken however, to define which list measures assess memory, and which assess learning. In this thesis, the ability to immediately recall information based on a single presentation of material is operationalised as *immediate memory*. Although some authors refer to Trial 1 as a measure of immediate *span* (eg. (Lezak, 1995; Mitrushina, Boone &

D'Elia, 1999; Spreen & Strauss, 1998) the current thesis reserves the use of span for tasks such as digit or word span. Thus, Trial 1 assesses the acquisition of newly presented information, and is therefore a measure of initial encoding (Lezak, 2004). Consistent with Miller's (1956 *Magic Number 7±2* a reasonable score for Trial 1 is between 5 and 9 items for healthy control participants (Salmon & Squire, 2009; Woodard, 2006). Recall performance over the five trials of the AVLT and CVLT reflects the ability of the examinee to increase the number of words recalled beyond their immediate memory score. This increase in performance reflects learning and the change of performance over the five trials is referred to as the learning curve. The learning curve reflects the rate of encoding and consolidation, and is quantified by Total Learning (the sum of words recalled across all five trials) and by Learning Over Trials (calculated as Total Learning – 5 * Trial 1). Total Learning has been demonstrated to show the best test-retest reliability of the summary scores, however Learning Over Trials corrects for baseline differences on Trial 1 and is more sensitive to variations in cognitive ability, therefore providing greater diagnostic utility (Tierney et al., 1994; Vakil, Blachstein & Sheinman 1998; Woodard, 2006). Although Total Learning is purported to measure rate of encoding and consolidation, the learning curve is affected by immediate memory, storage ability and retrieval efficiency and as such is a measure of multiple hypothetical memory components. When attempting to discriminate between components of memory using list learning tasks it is therefore necessary to consider the interdependence of each measure (for example, low recall scores for the learning rates might reflect an encoding deficit, or it might reflect poor retrieval ability). Rate of forgetting also impacts on the learning curve, and could be due to interference, inadequate retrieval cues resulting in retrieval failure or decay of the memory trace (poor consolidation). Forgetting is not reduced by the number of times a word is presented to be learned (Rubin & Wenzel, 1996; Slamenka & McElree, 1983) however as the number of successful retrieval attempts increases, forgetting is less likely to occur (Karpicke & Roediger, 2008). Examining participant forgetting patterns can provide information about whether they are failing to recall words that had previously been recalled once or twice (indicating the word has not been adequately consolidated) or whether they are failing to recall words that have previously been recalled several times consecutively, indicating forgetting. This is a purer measurement of forgetting, and

can help to differentiate between whether poor consolidation or higher incidence of forgetting is contributing to the overall learning curve (Lezak, 2004).

Verbal declarative memory is not only dependent on how well information is encoded, but also on how durable the stored information is. The learning curve is comprised of inter-trial, short term consolidation in which participants build on the number of words recalled on the preceding trial. This is referred to as short term consolidation in the present thesis. The traditional storage concept refers to long term consolidation and retrieval processes, usually assessed via recognition or delayed free recall. The recognition test is commonly used to measure consolidation and storage, by counting the number of words that were correctly identified form the original list (hits). Recognition tests are useful as they assess whether words that were not available in the free recall test were stored. According to the *Working with Memory* model, simple recognition is the domain of the hippocampal component. Recognition is a *cue dependent* test, meaning that the cue (target word) should be sufficient for retrieval. When an external cue interacts with the memory trace in the hippocampus, the trace is made conscious. For example, when asked "was this word (eg. umpire) on the original list?" the cue automatically elicits the trace, and a response "yes" or "no" is relatively automatic in individuals with healthy memory function. Hippocampal volume has been shown to be related to recognition accuracy on the CVLT (Kramer et al., 2005), and as mentioned previously the MTL has been implicated in recognition performance in several lesion and fMRI studies.
Delayed free recall measures consolidation, retention over time and retrieval, and can be assessed by the number of words recalled after the delay. A measure of retrieval efficiency can be derived by comparing the delayed recall score with the number of recognition hits, which indicates how many stored words could be retrieved without cuing. Retention and consolidation can be assessed by comparing the delayed recall score with the final learning trial (Trial 5) which provides the number of words that were forgotten during the delay (Lezak, 2004; Woodard, 2006). In contrast to cue dependent tests such as recognition, free recall trials do not provide a cue that is sufficient for retrieval. The cue may provide a starting point for retrieval, for example, asking "tell me all the words you can remember from the list" provides a contextual cue, but the items need to be searched for in memory.

According to the Working with Memory model then, free recall involves both the frontal and hippocampal components for successful task performance. Consistent with this prediction by the model co-activation of the dorsolateral prefontal cortex and perihinal cortex (Staresina & Davachi, 2006) the prefrontal cortex and left MTL (Alkire, Haier, Fallon & Cahill, 1998; Johnson, Saykin, Flashman, McAllister & Sparling, 2001) and hippocampus and perihinal cortex (Strange, Otten, Josephs, Rugg & Dolan, 2002) have been significantly correlated with free recall performance.

The test variables of the CVLT and AVLT have been demonstrated to reliably and accurately reflect different memory constructs. In a factor analysis study of AVLT scores, Vakil and Blachstein (1993) reported a three factor structure, comprised of Acquisition, Storage and Retrieval. As would be expected, Trial 1 loaded on to the Acquisition factor, as did Learning rate (Trial 5 score minus Trial 1). Recognition performance accounted for the majority of variance on the Storage factor and Delayed recall had the highest loading on the Retrieval factor. Thus, at least when considered with regard to the mediationsist account of memory stages, the RAVLT appears to have adequate construct validity.

The Role of Serotonin in Memory- Evidence from Acute Tryptophan Depletion Studies

The past two decades has produced numerous human experimental studies indicating that serotonin is involved in memory and learning. For example, reduced serotonergic functioning may be responsible for memory deficits found for depressed persons, people with Alzheimer's disease, and normal cognitive decline for older adults (Hasselbalch et al., 2008;Lai et al., 2002; Newhouse, Tatro, Naylor, Quealey & Delgado, 2002; Porter, Lunn & O'Brien, 2003; Richter-Levin & Segal, 2003). In order to investigate the role of serotonin in cognitive processes of humans, several studies have used verbal list learning tasks similar to the RAVLT and the CVLT, in combination with a procedure called acute tryptophan depletion (ATD). ATD has been used to mimic the effects of reduced serotonin function in the brain. Tryptophan is an enzyme that is involved in serotonin synthesis in the brain, and is almost uniquely found in serotonin neuron terminals of the raphe system. The acute

tryptophan depletion (ATD) method is used to induce lowered serotonin neurotransmission in the brain. This method involves replacing normal dietary intake with a drink that contains no tryptophan, but several amino acids that compete with tryptophan for access through the blood brain barrier. This procedure typically reduces serotonin synthesis in the human brain by 60-80% within 4-7 hours after consumption of the drink (Booij, Van der Does & Riedel, 2003; Norra, 2007). CSF sampling in healthy volunteers have demonstrated an 80-90% reduction in tryptophan levels post drink (Carpenter, Anderson, Pelton et al. 1998; Williams, Shoaf et al. 1999) and a PET study involving healthy female volunteers reported that 5-HT_2 receptor binding showed a significant decrease following ATD (Yatham, Liddle et al., 2001). ATD challenge paradigms are used to examine cognitive performance following a reduction in serotonin release, and as such can be used to better understand the role of serotonin in memory. ATD produces an acute disruption to serotonin synthesis in the absence of neuronal damage (Riedel, 2004) and thus provides useful information on the effects of altered serotonergic functioning on cognition. Assuming that ecstasy use produces alterations to serotonin availability after the acute intoxication phase, ATD may also provide a model of serotonergic degeneration which may simulate the effects of ecstasy use.

Linking serotonin with memory processes; acute tryptophan depletion studies and list learning tasks

Several studies have investigated the effect of ATD on healthy participants using either auditory or "visual" verbal list learning, usually consisting of a list between 15 and 30 words. For "visual" verbal learning tasks, the words are presented visually, on a computer screen, rather than being read aloud as is the standard RAVLT/CVLT procedure. These studies assessed Immediate recall, Delayed recall and Recognition performance and all reported a decrease in plasma tryptophan levels relative to the placebo condition. Schotissen et al. (2006) reported a trend towards impairment on Immediate recall for healthy older adults, however the majority of studies including healthy participants with mean ages ranging from 21-41 found that ATD did not impair Immediate list recall (Evers et al., 2005; Harrison et al., 2004; Klaassen et al., 1999; Riedel et al., 1999; Sambeth et al., 2009 and Scmitt et al., 2000). Similarly for the RAVLT or a 30 item auditory verbal

learning test, no effect of ATD was found for Immediate recall (Haywood et al., 2005; Hughes et al., 2003 and Shansis et al., 2000) although Porter et al. (2005) and Merens et al. (2008) reported lower Immediate recall for healthy older adults and remitted depressed persons respectively.

With regard to Delayed recall, visually presented verbal learning studies have reported reasonably consistent deficits following ATD (Harrison et al., 2004; Klaassen et al., 1999; Riedel et al., 1999; Sambeth et al., 2009 and Scmitt et al., 2000) although Evers et al. (2005) failed to find a deficit for this measure. For tests of auditory verbal learning, a Delayed recall deficit was only reported for healthy older adults (Porter et al., 2005). Only three of these studies reported impaired delayed Recognition performance (Schotissen et al., 2006; Riedel et al., 1999; Scmitt et al., 2000) and no effects of ATD for Recognition were reported for the auditory learning tasks. The lack of consistency between visual and auditory list presentations may be due to a ceiling effect observed for 15 word lists (Uttl, 2005) or due to the auditory modality being differently affected by ATD (Mendelsohn, Riedel & Sambeth, 2009).

In a meta-analysis of nine ATD studies which used the visual verbal learning test, performance was compared for Immediate recall, Delayed recall and Recognition on word lists consisting of 15 and 30 items. Effect sizes (*f*) were calculated as the standardised mean difference between tryptophan present and tryptophan depleted conditions. For 15 item lists, ATD impaired Immediate ($f = .13$) Delayed ($f = .31$) and Recognition ($f = .18$) performance, and this impairment increased for the 30 item list: Immediate ($f = .32$) Delayed ($f = .48$) Recognition ($f = .32$). For both list lengths, ATD had a greater effect on Delayed recall compared with Immediate recall and Recognition, suggesting that long term consolidation and retrieval are most impaired by reduced serotonin availability. Overall, these ATD results are indicative of the effect of lowering of serotonin on encoding and consolidating processes in episodic memory (Sambeth, 2007).

Harrison et al (2004) used the same group of participants to test the effects of ATD and acute tyrosine/phenylalanine (ATP- which has been shown to acutely

reduce dopaminergic function) on a visually presented verbal learning task and a working memory task. ATD impaired delayed recall performance relative to both placebo and ATP, and ATP had no effect on any of the verbal learning measures, however impaired spatial working memory performance. These finding suggest that low brain serotonin impairs episodic memory performance, whereas lowered dopamine activity impaired working memory, and supports evidence that low serotonin and dopamine may have selective effects on memory due to their actions in temporal and frontal brain regions. Van der Veen et al. (2006) provided further evidence for altered serotonergic functioning impacting on hippocampal processing. During ATD, van der Veen et al. used fMRI to examine changes in brain activation during the encoding and retrieval phase of a visually presented verbal episodic memory task. During the encoding phase, participants were asked to indicate whether the word gave them an emotionally positive or negative feeling. The retrieval phase consisted of a recognition task with distractors. During the encoding phase, fMRI showed decreased activation of the right hippocampus during ATD, and no differences in brain activation during ATD in the retrieval phase. The authors suggested that reduced serotonin levels impaired activation of the hippocampus during the encoding phase, leading to impaired encoding and consolidation of verbal items.

ATD, and therefore attenuated CNS serotonin synthesis, appears to selectively disrupt visual verbal learning. Several studies failed to find an effect of ATD on non-verbal learning tasks, such as the Rey Complex Figure Task (Hughes et al., 2003; Kulz et al., 2007) Rey Visual Design Learning (Porter et al., 2003; 2005; and a pattern recognition task (Booij et al., 2005; Evers et al., 2005; Park et al., 1994; Porter et al., 2003 and Roiser et al., 2007). For spatial memory, one study reported healthy controls needed more trials to learn spatial locations on a spatial recognition task (Park et al., 1994) and another reported improved scores on an object relocation task for the immediate task, and no differences between ATD and placebo on the delayed version (Sambeth et al., 2009). Other spatial memory studies failed to find any differences between ATD and placebo (eg. Amin et al., 2006; Hughes et al. 2002; 2003) and short term memory, as assessed by Forward Digit Span has also

been shown to immune to effects of ATD (eg. Luciana et al., 2001; Porter et al., 2003; Shansis et al., 2000; Stewart et a., 2002).

The reported findings suggest that disruptions to the serotonergic system have a negative effect on the ability to recall verbal information. Specifically, the results show a limited effect of ATD on Immediate recall, however Delayed recall was often impaired following ATD, indicating a role of serotonin in the consolidation and retrieval of newly learnt verbal information. This type of impairment suggests that serotonin plays a key role in verbal episodic memory. Furthermore, as recreational use of ecstasy is sometimes associated with decreased SERT density and reduced activity in the hippocampal region of regular ecstasy users compared with non-users, and both serotonin and the hippocampus are believed to be crucial to the formation of new memoires, it might be expected that regular use of ecstasy may impair memory. Chapter 6 provides a systematic review of the ecstasy and memory literature with a view to identifying which measure; Immediate, Delayed or Recognition performance, is most impaired in recreational consumers of ecstasy.

Chapter 6

Study 1. A Quantitative Review of the Effects of Ecstasy use on Trial 1, Total and Delayed recall measures of Auditory Verbal Learning Tests.

As will be reviewed below, the ecstasy and memory research to date is fraught with inconsistencies with regard to methodology and results, with several studies reporting an ecstasy related verbal memory deficit and several not. One possible explanation for this inconsistency is that the standard list learning tasks used in these studies obscure the specific processes that may contribute to verbal memory deficits. Single studies are often subject to low statistical power and mixed comparison groups which potentially mask or inflate true effects. The present study aimed to overcome these limitations by providing both a qualitative and quantitative review of studies that have examined the effects of ecstasy on verbal memory using word list learning tasks. The chapter will describe the state of the ecstasy and memory literature to date and expand on this by reporting meta-analyses for three specific measures of verbal memory derived from standard neuropsychological tests of word-list learning; Trial 1, Total recall and Delayed recall. In the current study, Immediate Memory is operationalized as performance on Trial 1, consolidation and learning are assessed by Total recall and retention was defined as Delayed recall performance.

Evidence for ecstasy related deficits on word list learning tasks

The possibility that ecstasy use impedes memory ability has underpinned several studies attempting to identify neurocognitive consequences of ecstasy use. Several studies have recruited regular consumers of ecstasy and other illicit drugs and administered batteries of neuropsychological tests to identify whether cognitive deficits exist, and if so, their type and severity. The vast majority of this research has used standard neuropsychological measures of learning such as the Rey Auditory Verbal Learning Test (RAVLT) the California Verbal learning Tests (CVLT) and the Rivermead Behavioural Memory Test (RBMT) to examine differences in recall scores that may be associated with ecstasy use. In an attempt to better link the association between ecstasy use and memory impairment, some authors have used regression analysis to investigate whether there is a dose dependent relationship between a measure of ecstasy use, such as estimated lifetime dose or number of

tablets taken in the preceding six months, and a list learning measure. An alternative to using regression analysis to look for dose related effects of ecstasy on verbal memory scores is to build dose effects into the design by comparing people with arbitrarily defined "high" and "low" lifetime consumption.

Brown, McKone and Ward (2010) administered the CVLT and RAVLT to regular ecstasy users (estimated lifetime dose = 384) cannabis users and drug naïve controls. For the CVLT they reported a significant deficit for ecstasy users compared with the cannabis group and the non-drug using controls on Total words recalled and Delayed recall, and no significant differences between the cannabis group and the drug-free controls. For the RAVLT, there was no effect of ecstasy on Trial 1, and a small deficit on Total words recalled which failed to reach statistical significance after differences in estimated pre-morbid IQ were taken into account. There was no evidence of a dose related effect for the CVLT and RAVLT scores however. Brown et al. concluded that for relatively simple tasks such as free recall of unrelated words, ecstasy use has minimal impact on performance, but for more complicated tasks that require strategic organisation, such as the CVLT, ecstasy-related deficits are more apparent, possibly due to the requirement of engaging multiple brain regions, such as the hippocampus, frontal lobes and perceptual areas for successful performance. In contrast, Bedi & Redman (2008) also reported no significant differences in verbal memory performance for the RAVLT, however when the summary scores for the RAVLT (including Total recall, Delayed recall and recognition) were combined into a verbal memory factor, they found estimated lifetime ecstasy consumption predicted verbal memory, with higher use associated with lower RAVLT scores.

Alternatively, Halpern et al. (2010) failed to find an ecstasy related deficit on the CVLT. They studied the effects of ecstasy on the neurocognitive functioning of 52 regular ecstasy users (median occasions of use for ecstasy use = 43.5 and cannabis= 10) and 59 non-ecstasy using controls (median cannabis use = 1), all of whom had attended at least ten all-night dance parties and had experienced less than ten occasions of alcohol intoxication (defined as more than four standard drinks in four hours). Halpern et al. screened participants for surreptitious drug use, using hair, urine and breath samples at the time of testing. Results indicated small impairments for ecstasy users compared with non-users on Wechsler Memory Scale-III spatial

span forwards and digit span backwards. Unlike Brown et al. Halpern et al. failed to identify verbal memory deficits on the CVLT, with ecstasy users and non-users performing equivalently on Trial 1 and Total recall (delayed recall results were not reported). Halpern et al. also compared "moderate" users (defined as those with fewer than 50 occasion of ecstasy use) with "heavy" users (greater than 50 occasions) on CVLT measures and reported no differences between sub-groups, or between the entire ecstasy using sample (median use of ecstasy = 43, cannabis= 10) and 59 non-ecstasy using controls (median cannabis use = 1). Halpern et al., conclude that there were no marked cognitive deficits associated with ecstasy use and that ecstasy use does not appear to produce lasting neurotoxicity. Although these results seem to conflict with those reported by Brown et al. (2010), it is difficult to compare the studies directly due to the different characteristics of the ecstasy using sample. As Halpern et al., note, their sample have a relatively low exposure to ecstasy (only 6 of the 22 "heavy" users had lifetime occasions of use greater than 150) whereas the mean estimated lifetime dose in the Brown et al., study was 384. Further difficulty for comparisons arises from the differences in other illicit drug use between the studies, as the Halpern et al., cohort also had very few occasions of intoxication for cannabis and alcohol, something that is not typical for regular ecstasy consumers generally (Parrot, 2001).

These disparate methods and results typify the current state of the ecstasy and memory research; studies have used a variety of different methodological approaches, and as such it is not surprising that conflicting results arise. Some authors have argued that the memory deficits associated with ecstasy use are negligible once other drug use has been taken into account (eg. Bedi & Redman, 2008; Halpern et al., 2011) while others suggest regular ecstasy use is associated with memory deficits, possibly via its impact on serotonergic system function (eg. Blagrove et al., 2010; Indlekofer et al., 2009). When considering three measures of memory commonly derived from word lists; Trial 1 score, Total recall and Delayed recall, findings have generally been inconsistent. For example, an ecstasy related deficit relative to control participants has sometimes been reported for Trial 1 (Parrot & Lasky, 1998; Fox, Toplis, Turner & Parrott, 2001; Gouzoulis-Mayfrank et al., 2000) while others have not identified an ecstasy related effect (Thomasius et al.,

2003, 2006; Curran & Verheyden, 2003; McCardle, Luebbers, Carter, Croft & Stough, 2004; Bedi & Redman, 2008; Brown, McKone & Ward, 2009). Similarly, there is a combination of significant and non-significant results for Total recall scores, with several studies reporting an ecstasy related deficit on this measure (Bolla et al., 1998; Parrott & Lasky, 1998; Reneman et al., 2001b: Thomasius et al., 2003; Quednow et al., 2006; Thomasius et al., 2006; Reneman et al., 2006; Lamers, Bechara, Rizzo & Ramaekers, 2006; Schilt et al., 2008; Schilt et al., 2010) although a few have not (Reneman, Booij, Scmand, van den Brink & Gunning, 1999; Croft, Mackay, Mills & Gruzelier, 2001; Reneman, Majoi, Schmand, van den Brink & den Heeten, 2001; Simon & Mattick, 2002; Back-Madruga et al., 2003; Bedi & Redman, 2008; Brown et al., 2009). Finally, the results for delayed recall have been equally inconsistent, with some studies reporting a delayed recall deficit associated with increased ecstasy consumption (Reneman, Booij, Scmand, van den Brink & Gunning, 1999; Reneman, Majoi, Schmand, van den Brink & den Heeten, 2001; Reneman et al., 2001b; Fox, Toplis, Turner & Parrott, 2001; Curran & Verheyden, 2003; Thomasius et al., 2003; McCardle, Luebbers, Carter, Croft & Stough, 2004; Yip & Lee, 2005; Reneman et al., 2006; Thomasius et al., 2006; Schilt et al., 2010) and some not (Bolla et al., 1998; Gouzoulis-Mayfrank et al., 2000; Croft, Mackay, Mills & Gruzelier, 2001; Simon & Mattick, 2002; Back-Madruga et al., 2003; Lamers, Bechara, Rizzo & Ramaekers, 2006; Bedi & Redman, 2008).

Compared with research on unrelated word lists, the research on semantically related word lists is limited. With the exception of Fox, Parrott and Turner (2001), who reported no differences between ecstasy users and controls for immediate and delayed recall, all studies which have examined performance on semantically-related word lists have used the CVLT. For Trial 1 scores of CVLT, only three studies report results, all of which failed to identify an ecstasy related effect (de Sola et al., 2008; Halpern et al., 2004; 2010). For Total recall, two studies report an ecstasy related deficit (Semple et al, 1999; Brown et al., 2009) and four do not (de Sola et al., 2008; Halpern et al., 2004; 2010; Medina, Shear & Corcoran, 2005). For Delayed recall, de Sola et al. (2008) failed to find an ecstasy related deficit, however Medina, Shear and Corcoran (2005) and Brown et al. (2009) reported that ecstasy consumers recalled significantly fewer words than control participants after a delay.

To investigate whether impairments change after a period of abstinence from ecstasy, Thomasius et al. (2003) compared former ecstasy users (minimum 20 weeks abstinence) with current users and poly-drug controls. In this study, males and females from both former and current ecstasy using groups had very similar levels of ecstasy consumption, and the poly-drug controls were well matched for other substance use. Current use and abstinence was tested with hair analysis and the concordance rate was 95%. Results showed that former, but not current, users performed significantly worse on Total and Delayed recall of the RAVLT. Regression analyses showed that typical number of tablets taken per session best predicted Trial 1 RAVLT scores, and estimated lifetime consumption best predicted Delayed recall. Dose dependent effects for Delayed recall have also been reported by Fox, Toplis et al. (2001), Yip & Lee (2005). In addition Schilt et al. (2008) and de Sola et al. (2008) reported a dose related effect on CVLT recognition. Although Medina et al. (2005) and Bedi and Redman (2008) found the majority of ecstasy consumers scoring within the normal range for the CVLT and RAVLT respectively, both groups demonstrated that estimated lifetime ecstasy consumption significantly predicted verbal memory performance. For Total recall Reneman et al. (2006) reported that heavy ecstasy consumers (defined as more than 50 tablets prior to testing) scored significantly lower on RAVLT total and delayed recall compared with moderate users (less than 50 tablets prior to testing). Alternatively, de Sola et al. (2008) Brown et al. (2010) and Schilt et al. (2010) failed to find a dose related relationship between lifetime ecstasy consumption and verbal memory.

To overcome the methodological limitations of small and heterogeneous drug using samples and the large amount of seemingly disparate results within the ecstasy and cognition literature, several meta-analyses have been conducted to assess the effects of ecstasy on cognitive functioning. Meta-analyses have the advantage of using data from several studies that have investigated the same task variables, and thus the significance and magnitude of the effect can be pooled to reveal a more consistent pattern of results. Dose related effects can also be investigated with a large sample size. The following section reviews the ecstasy and cognition meta-analyses

to date, focusing on the verbal learning and memory domain. A summary of these studies is presented in Table 6.1 below.

Table 6.1
Summary of Previous Ecstasy and Memory Meta-analyses

Review	Cognitive Domain	Number of Studies	Effect Size	Dose Related Effect
Short Term Memory				
Verbaten (2003)	Short term memory	10	-1.15[a]**	ns
Laws and Kokkalis (2007)	Short term memory	25	-0.63[b]***	ns
Nulsen et al. (2010)[z]	Verbal short term memory	30	-0.40[c]	ns
Working Memory				
Nulsen et al. (2010)[z]	Verbal working memory	22	-0.37 [c]	sig*
Verbal Memory				
Laws and Kokkalis (2007)	Verbal memory	22	-1.00[b]***	ns
Rogers et al. (2009)[x]	Verbal memory-total recall	35	-0.34[a]***	ns
Kalechstein et al. (2007)[y]	Verbal learning and memory	23	-0.73[b]***	na
Zakzanis et al. (2007)	Learning and memory	21	-0.55[b]	sig**
Verbal Memory Delayed				
Rogers et al. (2009)[x]	Verbal memory-delayed recall	27	-0.36[a]***	ns
Verbaten (2003)	Long term memory	10	-1.36[a]**	ns
Laws and Kokkalis (2007)	Long term memory	19	-0.87[b]***	ns

Notes: All studies corrected for random effects. [a] Mean Effect Size [b] Cohen's d, [c] Hedges g, [x] compared with polydrug groups, [y] lenient analysis only, [z] all studies included, *** p<.001, ** p<.01, * p <.05, na = not assessed, ns = not significant
Verbaten (2003) Short Term Memory comprises: working memory and logical memory subtests of the Wechsler Memory Scale (WMS), prose recall test (immediate recall), Trial 1 recall for; Rivermead Behavioural Memory Test (RBMT), Verbal Learning Memory Test (VLMT), Coughlan list and design learning.

Table notes from previous page: Verbaten (2003) Long Term Memory comprises delayed word recall, delayed prose recall, delayed recall trial on; RAVLT, RBMT, Coughlan design and list learning, WMS logical memory
Laws and Kokkalis (2007) Verbal Short Term Memory comprises digit span forwards, logical memory, verbal paired associates, Trial 1 of RBMT, RAVLT, Coughlan List and CVLT.
Long term measures typically involved the delay trials of the tasks.
Kalechstein et al. (2007) did not include description of tasks included.
Nulsen et al. (2007) Verbal Short Term Memory comprises digit span forwards, immediate prose recall, paired word recall, letter span, match to sample and Trial 1 on verbal list learning tasks including; RBMT, CVLT German and English RAVLT.
Nulsen et al. (2007) Verbal Working Memory comprises digit span backward, Keep-track task, Verbal n-back task, Computation span, Letter-number sequence, mental counters, Reading span, Consonant updating.

Verbaten (2003) provided the first meta-analysis investigating the effects of ecstasy use on different cognitive domains. This review included ten studies that provided measures of short term and long term verbal memory and attention. After controlling for lifetime exposure to ecstasy (number of tablets taken) and cannabis use, the mean effect size (MES) indicated that ecstasy users performed one standard deviation below non-ecstasy using controls on measures of short term memory (d = -1.15, z = -4.52) . For long term memory, the mean effect size was also large (d = -1.36, z = -3.65) and remained significant after taking into account previous cannabis use. The short term memory domain included prose recall, list learning (total words recalled and Trial 1 recall for the RAVLT) and as such included both measures of short term memory span and verbal learning in the one domain. The long term memory domain however was limited to delayed recall of words and prose. Verbaten concluded that, based on the limited studies available at that time, ecstasy use leads to a decline in short term memory functioning however may not be detrimental to long term memory, which might be more vulnerable to cannabis use.

Laws and Kokkalis (2007) provided an extension to Verbaten's 2003 review and examined the extent of the effects of ecstasy use on short and long term memory and verbal and visual memory. This yielded four composites; verbal memory, visual memory and short term memory and long term memory, the latter two comprising both verbal and visual memory scores. For short term memory, Laws and Kokkalis reported a moderate effect size (d = -0.63, 95% *CI*: -0.91, -0.41) and a larger effect for long term memory (d = -0.87, 95% *CI*: -1.38, -0.45), although the short term and long term effect sizes did not differ significantly from each other. The effect size for verbal memory was very large (d = -1.00, 95% *CI*: -1.45, -0.59) and significantly larger than that obtained for visual memory (d = -0.27, 95% *CI*: -0.55, -0.03).

According to these results, ecstasy users performed an average of one standard deviation below non-ecstasy users on verbal memory tasks and also had poorer short and long term memory scores relative to controls, with long term memory producing the larger effect size. There was no significant dose related effect for cannabis or ecstasy. Laws and Kokkalis suggest that the non-significant effect of the extent of ecstasy use on memory performance may be attributable to the relationship being stepwise rather than linear, such that an initial (unknown) dose of ecstasy may have a large effect on memory and further consumption may be less important. Alternatively, the variability in estimations of consumption and pill content may also compromise the dose related analyses. Laws and Kokkalis' effect sizes were considerably smaller than those reported by Verbaten, and the authors suggested this was because their sample contained more studies and therefore provided a more reliable estimate of effect size.

To attempt to control for some of the methodological limitations of published cross-sectional ecstasy studies, Kalechstein et al. (2007) categorised 44 ecstasy and neurocognition studies as lenient or stringent based on their level of control for potential moderator variables such as premorbid IQ and education. Kalechstein et al. investigated five cognitive domains; attention, verbal learning and memory, non-verbal learning and memory, motor/psychomotor speed and executive functioning, however the specific tasks included in the analyses were not detailed. Verbal learning and memory had the largest effect size for both the stringent (d = -0.85, 95% *CI*: -0.32, -1.34) and lenient analyses (d = -0.73, *95% CI*: -0.44, -1.01), and attention/concentration had the smallest effect size across both analyses, although even for this domain ecstasy use was still associated with a performance deficit equivalent to one third of a standard deviation below controls. Comparisons of effect sizes between the lenient and stringent analyses showed no significant differences, suggesting that the ecstasy related impact on cognition is observable regardless of the level of control for potential confounds. Based on these results Kalechstein et al. argued that the cognitive deficits associated with ecstasy use are not equal across different domains, with verbal learning and memory, information processing speed and executive functioning being most vulnerable to negative consequences of ecstasy use. According to Kalechstein et al. this pattern of deficits is consistent with a

"frontal-subcortical" typology. In the Kalechstein et al study, dose-related effects were not examined, and it was unclear whether the control participants were poly-drug users, drug naive or both, thus it is difficult to ascertain whether the effect sizes are attributable to ecstasy consumption.

Zakzanis, Campbell and Jovanovski (2007) performed meta-analyses to investigate the effects of ecstasy consumption across seven neuropsychological domains; learning and memory, verbal comprehension, processing speed, attention and concentration, executive function, perceptual organisation and motor skill. Zakzanis et al. included both poly-drug and drug naive controls, although if a study included both groups the former was given preference. Consistent with Kalechstein et al. (2007) the effect of ecstasy use was largest for learning and memory (*d* = -0.55, 95% *CI*: -0.76, -0.33), followed by verbal comprehension (*d* = -0.36, 95% *CI*: -0.54, -0.17) and processing speed (*d* = -0.33, 95% *CI:* -0.57 – 0.09). Unlike Verbaten (2003), and Laws and Kokkalis (2007) who failed to identify any dose related effects on memory performance, Zakzanis et al. reported a significant negative relationship between total lifetime ecstasy consumption and learning and memory scores.

In an updated meta-analysis, Nulsen, Fox and Hammond (2010) aimed to determine whether the effects of ecstasy use differ for short term and working memory in the verbal and visual domains. The measures for verbal short term memory included digit span forward, letter span, immediate recall subtest of the Rivermeand Behavioural Memory Test (RBMT), Trial 1 and Total recall of the RAVLT and CVLT. For verbal working memory, the tasks included digit span backwards, computation span, sentence span, n-back tasks and keep-track tasks. The magnitudes of the effect sizes for ecstasy users compared with poly-drug controls were largest for visuospatial working memory (*g* = -.60, 95% *CI* = -0.85, -0.36) and smallest for visuospatial short term memory (*g* = -0.25, *CI*: -0.49, -0.02). For verbal memory, the effect of ecstasy use was small for both short term (*g* = -0.40, 95% CI: -0.59, -0.21) and working memory (*g* = -0.37, 95% *CI*: -0.51, -0.23). Although the effect sizes across the four domains did not differ significantly from one another, self-reported lifetime ecstasy consumption only significantly predicted the effect size for the working memory domains. These results suggest that it is not the verbal or

visual nature of information that is differentially effected by ecstasy use, but rather the nature of the information processing. Thus, short term retention in either the verbal or visual domains is negatively impacted by ecstasy use to a small extent, however retention of, and working memory type manipulation of information is most impaired by ecstasy use.

In a comprehensive meta-analysis of published studies addressing ecstasy use and cognition, Rogers et al. (2009) reviewed studies that had included measures of Total recall and delayed verbal memory performance. The analysis included 46 different outcome measures, the most common being the RBMT, followed by the RAVLT and digit span backwards. The effect measures were expressed as standardised mean difference scores, and for verbal memory (*d* = -0.33, 95% *CI*: -0.47, -0.22) indicated that on average ecstasy users' memory performance was one third of a standard deviation worse than poly-drug controls. When compared with drug naive individuals, the magnitude of the effect increased to -0.85 (95% *CI*: -1.03, -0.67) indicating that immediate verbal memory performance among ecstasy users is almost one standard deviation below that of drug free controls. For Delayed recall measures, results showed that long term memory scores for ecstasy users were also one third of a standard deviation lower than poly-drug controls (*d* = -0.36, 95% *CI*: -0.49, -0.22) . When compared with drug naive controls, the effect increased to over one standard deviation of difference between scores (*d* = -1.11, 95% *CI*: -1.99, -0.23). Yip and Lee's (2005) unique study comparing 100 ecstasy-only users (reported to have no exposure to other illicit drugs and minimal exposure to alcohol and tobacco) with 100 drug naïve controls was an outlier in the meta-analysis, however when it was removed from the analysis the ecstasy effect was still large (-0.72, 95% *CI*: -0.91, -0.52). Rogers et al. failed to identify a dose related effect for ecstasy however.

Summary

In contrast to the individual studies of ecstasy related memory deficits, for which inconsistencies are the norm, all the meta-analytic reviews to date have reported significant verbal memory performance decrements for ecstasy users relative to polydrug and/or drug naive controls. The magnitude of the effect sizes

differs considerably across analyses however, and not all have identified dose related effects. Verbaten (2003), Laws and Kokkalis (2007) and Zakzanis et al. (2007) all reported verbal learning and memory as being the cognitive domain most impaired by ecstasy use. Alternatively, Nulsen, Fox and Hammond (2010) suggest that it is not a specific domain that is impaired by ecstasy consumption, but rather, more complicated cognitive processes such as mental manipulation of information that are compromised by ecstasy consumption. These analyses have the advantage of including a large number of studies and participants to evaluate the effects of ecstasy on memory, and have shown clear verbal memory deficits for ecstasy users.

A limitation of the meta-analyses reported is that they are unable to determine which specific memory processes are being impaired by ecstasy use. List learning tasks for example, comprise several memory processes, such as encoding, consolidation, retrieval, strategising and rehearsal. Many meta-analyses have combined Trial 1 recall with Total recall as a measure of verbal learning, therefore combining scores for immediate memory with learning over multiple trials. Factor analysis has shown that recall on Trial 1 is statistically different from recall based on Total RAVLT scores (Johnstone, Vieth, Johnson & Shaw, 2000; Vakil & Blachstein, 1993) and that Trial 1, but not subsequent trials, positively correlates with self-reported memory problems (Krch, Sumowski, DeLuca & Chiaravalloti, 2011) suggesting that these variables should be treated separately in meta-analyses. Similarly, several meta-analyses included prose recall, such as the Rivermead Behavioural Memory Test and list learning in the same measure. This is not a useful approach as a story allows for greater depth of processing (Craik & Lockhart, 1972) more scope for visualisation and contains more recall cues to recall items than a list of unrelated words, and as such the two tasks require different cognitive strategies for successful performance. Thus, because combining task variables in this manner makes the meaning of a "verbal learning and memory" impairment difficult to interpret, studies using the RBMT as the memory measure were excluded from the meta analysis.

Although the existing literature provides no clear pattern for which specific measures of list learning are more likely to be affected by ecstasy use, further

investigation of the outcome measures using meta-analysis can provide a more meaningful summary of ecstasy related deficits. With this in mind, Rogers et al. (2009) performed a meta-analysis with 8 studies that included data for Total recall and 7 for Delayed recall of the RAVLT. For Total recall, ecstasy users recalled an average of four words less than poly-drug controls (d = -3.9, 95% CI: -7.12, - 0.70) and performed at an average of half a standard deviation below the normative population aged between 20 and 29. For Delayed recall ecstasy users recalled an average of one word less than polydrug controls (d = -1.04, 95% CI: -1.6, -0.48 and again performed at one standard deviation below the normative population for this measure. There was no evidence for a dose dependent effect, although Rogers et al. noted there was a trend for worse performance on Total recall to be associated with longer duration of use.

The present study aimed to provide a quantitative review of studies that have examined the effects of ecstasy on verbal memory using word list learning tasks. Importantly, it separates the domain of verbal memory into three dependent variables that are commonly reported in the literature; Trial 1, Total recall and Delayed recall. This procedure allows possible differences in the ecstasy related effects to be observed, which can in turn inform about which memorial processes are impaired or spared following ecstasy use. The current meta-analysis expands on Rogers et al. (2009) meta-analysis, by including studies of verbal learning that reported Trial 1, Total recall and Delayed recall outcome measures. Of the thirteen studies that reported results for Trial 1 of the RAVLT, only three identified an ecstasy-related deficit. Trial 1 limits the use of extra cognitive processes such as imagery and strategic organisation to assist with encoding and retrieval and is therefore a more pure measure of immediate memory. Thus, performance on Trial 1 is a useful addition to a meta-analysis as it can inform as to whether ecstasy related memory deficits are in part mediated by limited memory span for words.

Method

The electronic database Psych. Info was searched using the terms *ecstasy* OR *MDMA* and *cognition* to identify publications up until June, 2013 (see Figure 6.1). Publications other than peer reviewed scholarly articles were excluded and the search was also restricted to English language publications with a human population. The search term *memory* was then used to search within these publications. The remaining abstracts were scanned and publications that focused solely on executive functions, working memory, visuospatial memory, prospective memory and studies that examined the acute effects of ecstasy were also excluded. The method sections of the remaining studies were scanned and only included if means and standard deviations for ecstasy users and non-users on a word list learning task that was comparable to the RAVLT were reported. This meant that studies that used the Rivermead Behavioural Memory Test, paired associated tasks and later trials of selective reminding tasks as tests of verbal episodic memory were excluded. Of the remaining 23 studies that were included in the analysis, the tasks were; the RAVLT (or a non-English equivalent: 18 studies), CVLT (four studies) one novel word list learning task consisting of non-semantically related words, and Trial 1 of the Bushke Selective Reminding Test (BSRT, one study). Trial 1 of the BSRT consists of free recall of 12 non-semantically related words. A summary of the included studies is presented in Table 6.3 at the end of this chapter.

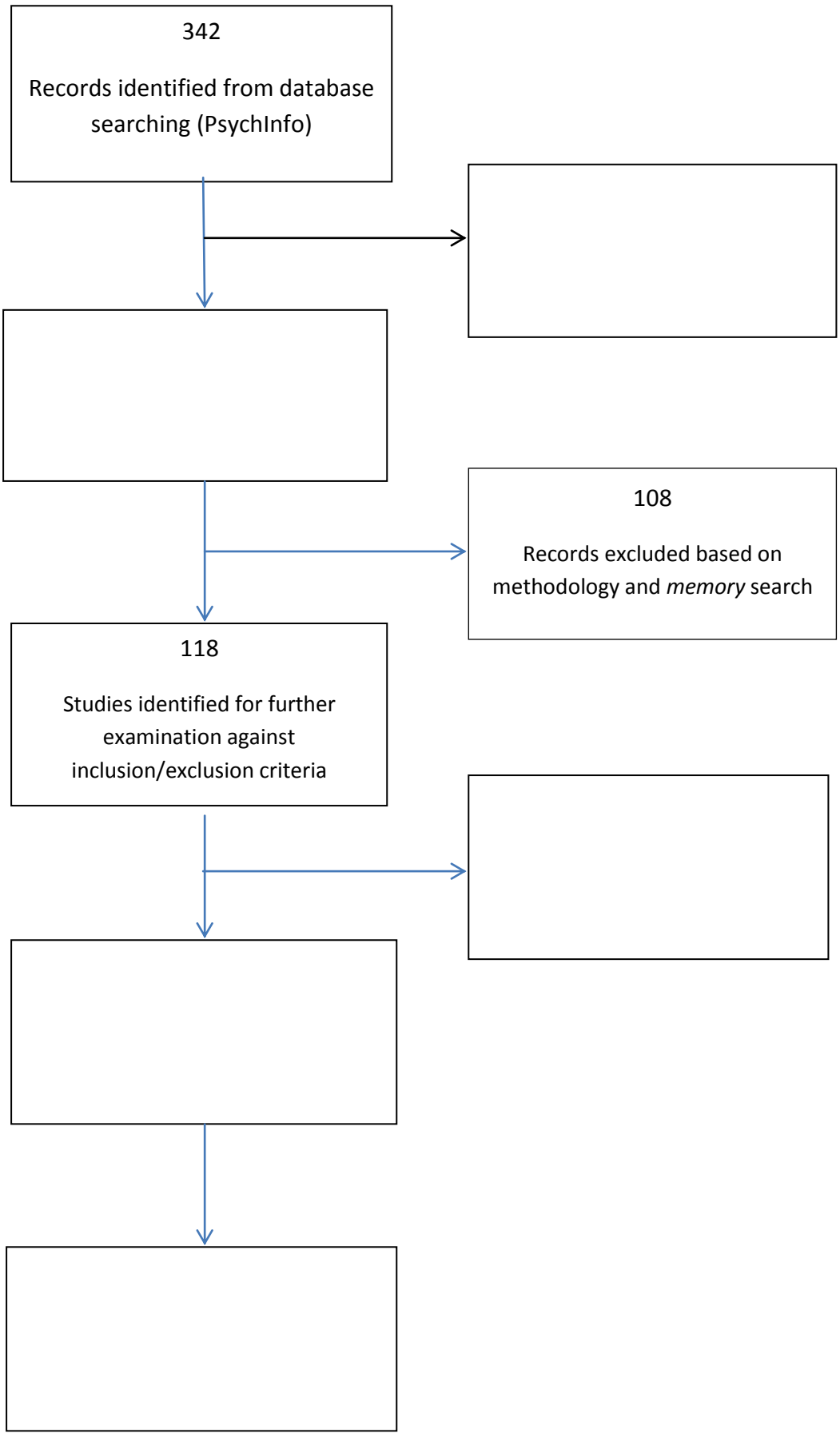

Figure 6.1. Flow diagram of the literature search process.

In studies where ecstasy consumers were categorised into light, moderate and heavy users, the categories were combined. In one study (Fox, Parrott & Turner, 2001) ecstasy consumers were classified based on whether they had reported significant problems related to their ecstasy use, such as experiencing affective distress or cognitive problems. In this instance, a conservative approach was taken and only the non-problem group was included to prevent the possible impact of prescribed psychotropic medications or low task motivation on the results. Because this study investigates the effects of current ecstasy use on memory performance, groups of ex-ecstasy users were also excluded from the analysis.

Numerous studies included control participants who had no or very little exposure to illicit drugs, and as such could be classified as drug naive. As ecstasy users typically use other illicit drugs such as amphetamines, cocaine or cannabis, comparing ecstasy users with drug naive persons is not ideal, as it allows differences in memory scores to be attributable to substances other than ecstasy (Verdejo-Garcia, Lopez-Torrecillas, Gimenez & Perez-Garcia, 2004). Comparing with other drug using individuals also means there is some level of control for the potentially confounding effect of other drug use. Therefore, two sets of analyses were undertaken, one analysis which included drug naive participants, and one that excluded them.

Effect sizes were calculated to identify the degree of ecstasy related impairment relative to controls on Trial 1, Total recall and Delayed recall measures. Analysing these measures separately avoids the problem where data that is indicative of different processes is combined to produce a summary main effect of ambiguous meaning (Lipsey & Wilson, 2001; Borenstein et al., 2009). For example, combining scores on Trial 1 and Total recall is questionable because these measures are thought to underlie different memory processes. The current meta-analysis therefore aimed to identify the specific measure of verbal learning most affected by ecstasy use.

Analyses

Meta-analyses were performed using the means, standard deviations, and group sizes (*n*) for scores on Trial 1, Total recall and Delayed recall. The measure of

effect size chosen was Hedges' *g*, as this corrects for problems arising from small sample sizes. Due to the heterogeneous nature of drug research participants, and as recommended for current meta-analytic studies, a random effects model was chosen *a-priori* (Borenstein et al., 2009). Publication bias, whereby peer-reviewed journals tend to publish more studies with statistically significant results, can prevent a true effect from being identified due to a number of unknown results being excluded from analyses. This was addressed by reporting Rosenthal's fail-safe *N* (*NFS*) for each analysis. This statistic provides an estimate of the number of studies required to render a result non-significant. A true effect, rather than a result due to publication bias is more likely to be found with a higher NFS (Laws & Kokkalis, 2007; Nulsen, Fox & Hammond, 2010). Effect sizes were classified according to Cohen's (1969) recommendation; $g = 0.20 – 0.49$ is a small effect, $g = 0.50 – 0.79$ is a medium effect and $g > 0.80$ is a large effect. In addition to effect sizes, meta-regression was performed using estimated lifetime ecstasy consumption (number of tablets) as a predictor of effect sizes where this data was available. Meta-analyses were conducted using Comprehensive Meta-Analysis (CMA 2.0) software.

Results

Effect sizes and 95% confidence intervals for the effect of ecstasy use on Trial 1, Total recall and Delayed recall for all studies are presented in Figures 6.2, 6.3 and 6.4 respectively. There was a small effect of ecstasy use for Trial 1 recall scores compared with drug naive and poly-drug controls. The ecstasy related deficits for Total and Delayed recall were moderate, with ecstasy associated with a performance decrement of over half a standard deviation relative to controls. All effect sizes were significant ($p < 0.01$).

Table 6.2 summarises the results for the analyses that included and excluded drug naive participants. As can be seen, removing drug naive participants from the analysis made little difference to the magnitude of the effect sizes, with the exception of Trial 1. The small effect for Trial 1 decreased further, indicating that the effect of ecstasy use on short term memory span is negligible. This was confirmed by the very low NFS value, which indicates only 9 studies would be required to produce a non-

significant ecstasy related effect for Trial 1. Using estimates of mean lifetime ecstasy consumption (reported in Table 6.3 when available) meta-regression failed to identify any significant relationship between ecstasy consumption and effect size for Trial 1(β = .00019, *SE* = .00060, *z* = .32, *p* = .75).

For Total recall, the removal of drug naive participants had very minimal effect on the magnitude of the ecstasy effect, with the effect size remaining in the moderate range for this measure, and although the NFS decreased, it was still high relative to the number of studies included (Table 6.3). Meta-regression identified the relationship between effect size and ecstasy consumption to be non-significant (β = .00041, *SE* = .00069, *z* =.96, *p* = .33).

For Delayed recall, as can be seen in Figure 6.4, the Yip and Lee (2005) study is anomalous compared with the other studies and consequentially this study was removed from the meta-analysis. Removal of this study reduced the magnitude of the effect from *g* = -.98 to *g* = -.67 however removal of the other studies that included a drug naïve comparison (Bedi & Redman, 2008; Reneman, Booij et al., 1999; Schilt et al., 2010) made little difference to the effect size (*g* = .60). Yip and Lee reported that participants in their study had no exposure to illicit drugs other than ecstasy and minimal exposure to tobacco and alcohol. Nearly two-thirds of potential participants were excluded from this study, leaving only relatively new users in the sample. This pattern of use is thus quite different from many studies, who typically recruit regular, as opposed to novice, ecstasy users. Although it is unclear why this exclusion appears to have inflated the effect size, this study is not representative of ecstasy studies generally (Rogers et al., 2009). There was evidence of a dose related effect for Delayed recall, with meta-regression including estimates of lifetime ecstasy consumption from seven studies showing a significant linear relationship between ecstasy consumption and effect size (β = .0008, *SE* = .00036, *z* = 2.38, *p* = .02).

Study name	Subgroup within study	Comparison	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Gouzoulis-Mayfrank 2000	Ecstasy-polydrug	Cannabis	VLMT Trial 1	0.443	0.267	0.071	-0.080	0.966	1.660	0.097
Halpern et al. 2010	Ecstasy-polydrug	Polydrug-no ecstasy	CVLT trial 1	-0.039	0.189	0.036	-0.409	0.331	-0.206	0.836
McCardle et al. 2004	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT trial 1	0.278	0.347	0.120	-0.402	0.958	0.801	0.423
Curran et al 2003	Ecstasy-current users	Polydrug-no ecstasy	Bushke trial 1	0.147	0.247	0.061	-0.338	0.631	0.593	0.553
Halpern et al. 2004	Ecstasy-only	Drug naive	CVLT trial 1	0.211	0.320	0.102	-0.416	0.838	0.660	0.509
Brown et al. 2010	Ecstasy-polydrug	Drug naive	RAVLT trial 1	2.242	0.317	0.101	1.621	2.864	7.073	0.000
Thomasius et al. 2006	Ecstasy-current	Drug naive	RAVLT trial 1	0.401	0.388	0.151	-0.361	1.162	1.032	0.302
de Sola Llopis et al. 2008	Ecstasy-polydrug	Cannabis	CVLT trial 1	0.241	0.263	0.069	-0.274	0.757	0.918	0.359
Fox, Parrot & Turner, 2001	Ecstasy-polydrug	Polydrug- no ecstasy	Trial 1	-0.215	0.311	0.097	-0.824	0.394	-0.691	0.489
Golding, Groome et al. 2007	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT Trial 1	0.142	0.310	0.096	-0.466	0.751	0.459	0.646
Wagner et al., 2012	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT Trial 1-follow up	0.237	0.256	0.066	-0.265	0.739	0.926	0.355
				0.359	0.180	0.032	0.007	0.712	1.997	0.046

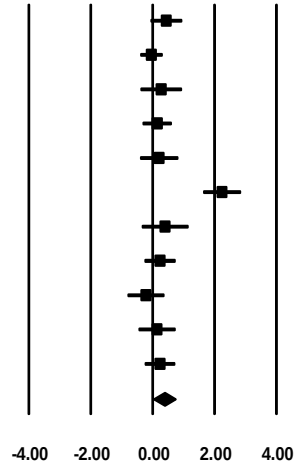

Figure 6.2. Effect sizes and confidence intervals for all Studies included in Trial 1 analysis

Figure note: Positive (+) values indicate an ecstasy related deficit.

Study name	Subgroup within study	Comparison	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Schilt, deWin 2007	Ecstasy-polydrug	Polydrug (<10 ecstasy)	RAVLT total	0.737	0.250	0.063	0.247	1.228	2.945	0.003
Halpern et al. 2010	Ecstasy-polydrug	Polydrug-no ecstasy	CVLT total	0.107	0.189	0.036	-0.264	0.477	0.566	0.572
Quednow et al 2006	Ecstasy-polydrug	Cannabis	RAVLT total	1.161	0.344	0.119	0.486	1.836	3.371	0.001
Croft et al. 2001	Ecstasy and cannabis	Cannabis	RAVLT total	-0.088	0.372	0.139	-0.818	0.641	-0.237	0.813
Halpern et al. 2004	Ecstasy-only	Drug naive	CVLT total	0.206	0.320	0.102	-0.421	0.833	0.644	0.520
Medina et al. 2005	Ecstasy-polydrug	Cannabis	CVLT total	0.653	0.285	0.081	0.095	1.211	2.294	0.022
Schilt et al. 2010	Ecstasy-old	Drug naive	RAVLT total	1.161	0.350	0.122	0.476	1.847	3.319	0.001
deSola et al. 2008	Ecstasy-polydrug	Cannabis	RAVLT total	0.059	0.374	0.140	-0.673	0.791	0.158	0.874
Brown et al. 2010	Ecstasy-polydrug	Cannabis	CVLT total	2.340	0.320	0.102	1.713	2.966	7.319	0.000
Lamers et al. 2006	Ecstasy and cannabis	Cannabis	RAVLT total	0.106	0.385	0.148	-0.648	0.860	0.275	0.783
Thomasius et al. 2006	Ecstasy-current	Drug naive	RAVLT total	0.812	0.401	0.160	0.027	1.597	2.028	0.043
Reneman, Schilt et al. 2006	Ecstasy-high	Polydrug-no ecstasy	RAVLT total	1.589	0.391	0.153	0.823	2.356	4.064	0.000
Reneman, Schilt et al. 2006	Ecstasy-moderate	Polydrug-no ecstasy	RAVLT total	1.092	0.396	0.157	0.317	1.868	2.760	0.006
Reneman, Majoi et al. 2001	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT total	0.922	0.515	0.266	-0.088	1.932	1.789	0.074
de Sola Llopis et al. 2008	Ecstasy-polydrug	Cannabis	CVLT total	0.518	0.266	0.071	-0.004	1.040	1.946	0.052
Hanson & Luciana, 2010	Ecstasy-polydrug	Drug naive	RAVLT Total	0.320	0.231	0.053	-0.133	0.772	1.384	0.166
				0.714	0.159	0.025	0.402	1.027	4.479	0.000

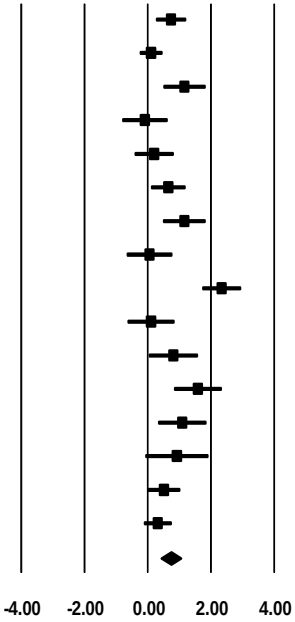

Figure 6.3. Effect sizes and confidence intervals for all Studies included in Total recall analysis
Figure note: Positive (+) values indicate an ecstasy related deficit.

Study name	Subgroup within study	Comparison	Outcome	Hedges's g	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Gouzoulis-Mayfrank 2000	Ecstasy-polydrug	Cannabis	VLMT delay	0.435	0.267	0.071	-0.088	0.958	1.631	0.103
Schilt, deWin 2007	Ecstasy-polydrug	Polydrug (<10 ecstasy)	RAVLT delay	0.913	0.255	0.065	0.414	1.413	3.586	0.000
McCardle et al. 2004	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT delay	0.389	0.349	0.122	-0.294	1.072	1.115	0.265
Croft et al. 2001	Ecstasy and cannabis	Cannabis	RAVLT delay	-0.088	0.372	0.139	-0.818	0.641	-0.237	0.813
Reneman, Booij et al. 2000	Ecstasy-polydrug	Drug naive	RAVLTdelay	2.255	0.674	0.454	0.934	3.576	3.345	0.001
Yip & Lee 2005	Ecstasy-only	Drug naive	AVLT delay	5.767	0.321	0.103	5.138	6.396	17.970	0.000
Schilt et al. 2010	Ecstasy-old	Drug naive	RAVLT delay	1.138	0.349	0.122	0.455	1.822	3.264	0.001
Bedi & Redman 2008	Ecstasy-polydrug	Drug naive	RAVLT delay	0.038	0.215	0.046	-0.384	0.460	0.176	0.860
Brown et al. 2010	Ecstasy-polydrug	Cannabis	CVLT delay	3.268	0.377	0.142	2.529	4.007	8.665	0.000
Lamers et al. 2006	Ecstasy and cannabis	Cannabis	RAVLT delay	-0.270	0.386	0.149	-1.027	0.487	-0.699	0.484
Thomasius et al. 2006	Ecstasy-current	Drug naive	RAVLT delay	0.764	0.399	0.159	-0.018	1.545	1.915	0.055
Reneman, Schilt et al. 2006	Ecstasy-high	Polydrug-no ecstasy	RAVLT delay	1.223	0.372	0.138	0.494	1.951	3.289	0.001
Reneman, Schilt et al. 2006	Ecstasy-moderate	Polydrug-no ecstasy	RAVLT delay	0.848	0.385	0.148	0.094	1.603	2.203	0.028
Reneman, Majoi et al. 2001	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT delay	1.059	0.524	0.275	0.032	2.087	2.021	0.043
de Sola Llopis et al. 2008	Ecstasy-polydrug	Cannabis	CVLT delay	0.353	0.264	0.070	-0.165	0.870	1.336	0.182
Fox, Parrot & Turner, 2001	Ecstasy-polydrug	Polydrug- no ecstasy	Delayed recall	-0.247	0.311	0.097	-0.857	0.363	-0.794	0.427
Golding, Groome et al. 2007	Ecstasy-polydrug	Polydrug-no ecstasy	RAVLT delay	0.157	0.310	0.096	-0.452	0.765	0.505	0.613
Hanson & Luciana, 2010	Ecstasy-polydrug	Drug naive	RAVLT Delay	-0.129	0.230	0.053	-0.579	0.322	-0.560	0.576
				0.979	0.347	0.120	0.300	1.658	2.824	0.005

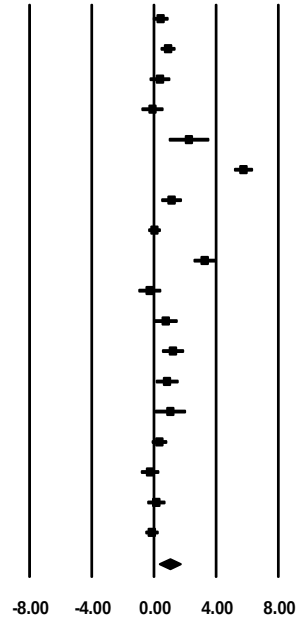

Figure 6.4. Effect sizes and confidence intervals for all Studies included in Delayed recall analysis
Figure note: Positive (+) values indicate an ecstasy related deficit

Table 6.2

Summary Statistics for Trial 1, Total and Delayed Recall Meta-analyses

	Hedges' *g* (SE)	95 % confidence Interval	*Z*	*Zp*	*Q*	*Qp*	*NFS*
All studies (N = 1422*)							
Trial 1	-.36 (.47)	.007 - .72	1.99	.046	44.68	<.001	35
Total recall	-.71 (.16)	.42 - 1.03	4.48	001	60.70	<.001	297
Delayed recall	-.98 (.35)	.30 – 1.66	2.82	.005	353.64	<.001	601
Delayed recall- Yip and Lee (2005) excluded	-.67 (.19)	.27 – 1.04	3.34	.001	98.81	<.001	230
Without Drug Naïve (N = 1012)							
Trial 1	-.15 (.09)	.03 - .33	1.67	.094	4.35	.82	9
Total recall	-.71 (.18)	.35 – 1.06	3.89	<.001	56.79	<.001	212
Delayed recall	-.60 (.20)	.21 - .99	3.01	.003	79.24	<.001	168

* Ecstasy users *n* = 723, Control *n* = 699. + Ecstasy users *n* = 514, Control *n* = 498.

Discussion

This meta-analysis has investigated three measures of verbal list learning in an attempt to identify which aspect of the task is most affected by ecstasy use. This was only achievable when studies reported group performance for each dependent variable rather than combining scores from multiple aspects of performance into a single overall score. This allows for a clear interpretation of the effect ecstasy use has on Trial1, Total and Delayed recall scores. The effect size measures showed that ecstasy users performed more poorly than non-ecstasy users on each list learning measure, and the magnitude of the effect was largest for Total recall, moderate for Delayed recall and small for Trial 1. Removal of the drug naïve comparisons so that studies comparing ecstasy users with poly-drug controls were only included made little difference to the magnitudes for the effect of ecstasy use on Total and Delayed recall. Given the current concerns raised in the literature regarding whether it is ecstasy or poly-drug use that is contributing to memory deficits frequently reported for ecstasy users, the comparison of ecstasy users with poly-drug consumers only is an important one as it provides support for an ecstasy specific deficit on the three measures of verbal memory that are most commonly reported in the ecstasy and cognition literature. Nulsen, Fox and Hammond (2010) and Rodgers (2009) also compared ecstasy users with poly-drug using controls in their meta-analyses and also reported small to moderate effects of ecstasy on global measures of verbal memory performance. Since comparisons between ecstasy and poly-drug users have been demonstrated to reduce estimates of effect sizes relative to ecstasy and drug naïve comparisons (Taylor, Greene, Morgan & Munafò, 2011) the finding that the magnitudes for Total and Delayed recall remained moderate in the current and previous studies suggests these effects are robust. A novel finding from the current study however, is the lack of ecstasy related effect for Trial 1 recall. This finding can provide greater clarity regarding the components of verbal memory that are spared by regular ecstasy use and can also better inform about the memory processes that are associated with an ecstasy related deficit.

Weak effects of ecstasy use on Trial 1 of verbal list learning tasks

Of the three measures examined in the current study, Trial 1 had the smallest effect size and the *NFS* of 9 indicates that only nine additional studies included in the analysis would be required to render the effect statistically non-significant. It is therefore possible that the effect for Trial 1 is a result of publication bias rather a true effect, and in either case the magnitude of a Trial 1 effect appears minimal in magnitude. Previous meta-analyses have not assessed the effect of Ecstasy on Trial 1, and individual studies have reported inconsistent results, with some earlier studies reporting a Trial 1 decrement (Parrot & Lasky, 1998; Fox, Toplis, Turner & Parrott, 2001; Gouzoulis-Mayfrank et al., 2000) and later studies finding no ecstasy related deficit (Thomasius et al., 2003, 2006; Curran & Verheyden, 2003; McCardle, Luebbers, Carter, Croft & Stough, 2004; Bedi & Redman, 2008; Brown, McKone & Ward, 2010). The low magnitude effect for ecstasy on Trial 1 in the current study is consistent with the later, but not earlier studies for this measure. The discrepancy in findings between earlier and later studies has recently been investigated by Taylor, Greene, Morgan and Munafò (2011). These authors used linear regression to ascertain the relationship between various study characteristics and estimates of effect sizes and found a significant relationship between increased year of publication and reduced effect size in the ecstasy and cognition literature (B = .04, 95% CI: .01 - .07, p = .024). They suggested there may have been an improvement in study quality over time, such as better control over the influence of drugs other than ecstasy that has reduced the likelihood of observing inflated effect sizes.

There is little consensus in the memory literature with regard to the component of memory that Trial 1 actually measures, with initial acquisition (Blachstein, 1993) simple attention span (Woodard, 2006) and immediate memory (Lezak, 2004) all being assessed by single trial word list recall. In contrast to Total and Delayed recall scores, which are derived after multiple list presentations and measure multiple processes, including inter-trial consolidation, storage and learning , Trial 1 performance assesses the capacity to acquire and retrieve information from a single trial and is thus more dependent on sub-vocal rehearsal of the items and maintaining them in short term memory for a brief amount of time. The trivial ecstasy effect for Trial 1 suggests that the phonological loop and initial encoding, or

processing of information to be stored, is largely un-impeded by regular ecstasy use. Further, the magnitude for Trial 1 means that initial acquisition of words is relatively equal between ecstasy users and non-users and is therefore not having a substantial impact on Total recall scores.

The effect of Ecstasy use on Total recall

Total recall measures the number of words recalled over multiple trials, and was the most commonly reported measure within the individual ecstasy and list learning studies, with several studies reporting an ecstasy related deficit for this measure (Bolla et al., 1998; Parrott & Lasky, 1998; Reneman et al., 2001b: Thomasius et al., 2003; Quednow et al., 2006; Thomasius et al., 2006; Reneman et al., 2006; Lamers, Bechara, Rizzo & Ramaekers, 2006; Schilt et al., 2008; Schilt et al., 2010) although the ecstasy group-means vary considerably. Based on 16 studies that included Total recall as a dependent variable, the current meta-analysis found a moderate effect of ecstasy on Total recall (g =-.71) in both the combined and the drug naïve excluded analyses.

Compared to Trial 1 findings, for which the effect of ecstasy use was weak, the ecstasy related effect was much larger as a consequence of the addition of multiple learning trials. Unlike Trial 1, which provides little scope for strategically organising input, multiple list presentations allow participants to build on the number of words initially encoded in Trial 1 and to engage in learning strategies that can facilitate consolidation and retrieval. Thus, the lack of a substantial Trial 1 deficit, coupled with the moderate ecstasy impairment for total words recalled informs us that for ecstasy users, it is not the initial acquisition of words that is impaired so much as the ability to build on the initial level of word acquisition efficiently, suggesting there is a verbal learning, rather than immediate memory deficit. Total recall is comprised of multiple cognitive components, and previous deficits for ecstasy users on this measure have been attributed to encoding problems (Ward et al., 2006) working memory or executive dysfunction (Gouzoulis-Mayfrank, 2000; Verbaten, 2010, Zakzanis et al., 2007) and retrieval and temporal lobe deficits (Daumann et al., 2005; Fox et al., 2002). Thus, Total recall relies on encoding, consolidation and retrieval processes, as well as organisational or semantic strategies

that enhance encoding and consolidation, and as such this measure provides limited insight into the specific cognitive processes that cause impairment.

The effect of ecstasy use on Delayed recall

The ecstasy related effect for Delayed recall was moderate (g = -.67) and this reduced slightly for the drug naive excluded analysis (g = -.60). This result is consistent with previous research identifying an ecstasy related effect on this measure (Reneman, Booij, Scmand, van den Brink & Gunning, 1999; Reneman, Majoi, Schmand, van den Brink & den Heeten, 2001; Reneman et al., 2001b; Fox, Toplis, Turner & Parrott, 2001; Curran & Verheyden, 2003; Thomasius et al., 2003; McCardle, Luebbers, Carter, Croft & Stough, 2004; Yip & Lee, 2005; Reneman et al., 2006; Thomasius et al., 2006; Schilt et al., 2010). This Delayed recall impairment may indicate that ecstasy users are either failing to retain previously learnt words (that is, they have forgotten more words than non-ecstasy users) or they have stored the words, but are unable to retrieve them. Due to the marked impairment on Total recall, it not possible to ascertain whether the Delayed recall deficit is associated with higher rates of forgetting for ecstasy users during the delay or whether it is consequence of their already low memory scores as identified on Total recall, which has impacted on the Delayed recall scores. A finer examination of the learning curve and recall consistency over the five trials of the RAVLT or CVLT could address this question directly.

The relationship between lifetime ecstasy consumption and measures of verbal memory

Although the current meta-analysis found minimal difference in magnitudes between the combined and drug naïve excluded analyses for Total recall, which supports the view that the observed effects are not due to poly-drug use, it is noteworthy that meta-regression examining the relationship between estimated lifetime consumption of ecstasy and Total recall scores failed to show a significant linear relationship between these variables. As the broad domain of verbal memory was compartmentalised into three measures in the current study, this reduced the number of studies available that had reported estimated lifetime consumption to seven for Total and Delayed recall and five for Trial 1. It is therefore possible that

the non-significant relationship between estimated ecstasy usage and Total recall scores reflects lack of adequate power for a meta-regression analysis (Borenstein et al., 2009). Nevertheless, the lack of dose dependence is broadly consistent with previous meta-analyses that have not found a significant relationship between ecstasy consumption and effect size for visual and verbal short and long term memory (eg. Laws and Kokkalis, 2007; Nulsen et al., 2010; Rogers et al., 2009 Verbaten, 2003). Nulsen et al. did find dose related effects for the working memory category of studies however, and there was significant dose related effect for the broad category of verbal learning and memory as reported by Zakzanis et al. (2007). Consistent with Rogers et al., the current meta-analysis found no relationship between ecstasy consumption and Total recall, however the present study did report a significant dose related effect for Delayed recall, where Rogers et al. did not.

Methodological considerations for ecstasy and memory research

It is difficult to draw comparisons between current and previous meta-analyses however, as the literature is fraught with inconsistencies in how ecstasy consumption is measured and reported. Most, but not all, of the cited research reports estimated lifetime dose (number of tablets) however there is a great deal of variability in how ecstasy use is quantified (eg. total occasions of use versus number of units used, number of pills consumed in the preceding six months, estimated lifetime dose, age of onset, usual number of tablets per session, maximum number of tablets taken per session). This array of potential dose-related predictors may make it difficult for a consistent dose related effect to emerge between studies. The characteristics and variability in patterns of drug use (cumulative and acute dosage, concomitant drug use, frequency of use) could have contributed to the variability in dose related findings also, particularly if bingeing or recency of use has a greater impact on cognitive performance than long term, occasional use (Fox, Parrott & Turner, 2001; Gouzoulis-Mayfrank & Daumann, 2006; Parrott, 2005). Other potentially important non-drug factors that can contribute to different cognitive outcomes include potential neuro-protection from concomitant cannabis use (Dumont et al., 2009), environmental factors (ambient temperature, noise, physical activity and dehydration) premorbid vulnerability (Parrott, 2006) and variations of the 5-HTT gene in the cognitive sequelae of ecstasy use (Fagundo et al., 2010;

Roiser, Rogers, Cook & Sahakian, 2006). Reliance on accuracy of participant's self-reports is also often cited as an unreliable estimate of use. Despite some misgivings however, procedures such as the Lifetime Drug-Use Interview (Czermak et al., 2005) have been shown to have adequate validity, reliability and test-retest reliability for research purposes (Czermak et al.; Darke, 1998; Harrison, Martin, Enev, & Harrington, 2007; Morgan, 2000).

Aside from differences in reporting conventions, establishing dose related effects is problematic due to the variability of MDMA content in pills sold as ecstasy, with some purity studies identifying consistent levels of MDMA in police seizure samples (eg. Cole et al., 2002; Simonsen et al. 2003; Parrott 2004; Giraudon and Bello 2007; Vogels et al. 2009) and others not (eg. Cheng et al., 2003). Many studies report fluctuating pill purity over time and identify several illicit substances other than MDMA in pills sold as ecstasy (Baggot et al., 2000; Morefield et al., 2011; Parrott, 2004; Tanner-Smith, 2006; Vogels et al. 2009), thus making it very difficult to establish whether neurocognitive effects are a result of MDMA use. Even when estimated ecstasy dose in 'numbers of pills' is recorded accurately, the discrepancies in pill constitution may mean that each unit of measurement fluctuates for each participant. Although establishing the exact pill content in retrospective ecstasy research is not possible, the majority of ecstasy users attempt to find out about pill content prior to ingestion (Johnston et al., 2006) and use physiological indicators such as nausea and teeth grinding to estimate the strength and purity of ecstasy tablets (Parrott, 2004). Participants' subjective ratings of MDMA-intoxication have been shown to be significantly correlated with MDMA plasma levels (Kolbrich et al., 2008) and higher MDMA pill content (Brunt, Koeter, Niesink, van den Brink, 2012). Overall, differences in drug consumption patterns, pill constitution, premorbid vulnerability and non-drug factors likely modulate the degree of memory impairment that can be attributed to dose-related ecstasy use.

Methodological problems associated with retrospective, cross sectional designs have been frequently discussed in the ecstasy (and other illicit drug) literature. Cross sectional designs possess several potential confounds that may not be distributed equally between ecstasy users and non-users, such as intelligence,

education and poly-drug use. Poly-drug use can be addressed in cross sectional designs by including participants with minimal exposure to other illicit drugs, however the majority of ecstasy users consume other substances and excluding other drug use can make the results less ecologically valid and make study sizes too small. Thus, including poly drug users, whether compared to drug naïve or ecstasy-only users, can undermine the confidence with which memory related effects can be attributed to ecstasy use alone. Moreover, poly-drug users or ecstasy-only users may have more occasional patterns of use than poly-drug ecstasy users (Degenhardt & Hall, 2010), which creates large discrepancies in lifetime drug use. Indeed, there was a degree of variability in poly-drug use within individual studies in the present meta-analysis. For example, Schilt et al. (2010) recruited ecstasy users with a mean lifetime use of 318 (SD = 511) grams of amphetamines, compared with 287 (799) grams for their poly-substance group. Alternatively, the ecstasy using group in Bedi and Redman's (2008) study used an average of 23.5 grams of amphetamines (SD= 68.4) compared with just 0.3 grams (22) for their poly-substance group. Bedi and Redman's groups were well matched for cannabis use however, with the ecstasy group using an average of 355.6 (SD = 616.9) joints in their lifetime, compared with 360.4 (634.2) for the poly-substance group. Despite the shortcomings of retrospective studies, the present and previous meta-analyses have the advantage of estimating effect sizes from a large population of ecstasy users and have shown moderate magnitude ecstasy related verbal memory deficits which remain moderate even after the removal of drug naïve controls.

Conclusion

Overall, this meta-analysis has found limited evidence of an ecstasy related effect for immediate memory, and moderate, robust effects of ecstasy on Total and Delayed recall. Previous research that has identified reduced Total and Delayed recall scores for ecstasy users have suggested these may be indicative of temporal lobe, and in particular hippocampal dysfunction (Fox et al., 2002; Gouzouilis-Mayfrank et al., 2003). More recent research has attempted to unravel the drivers of deficits on Total recall scores by including more specific list learning measures such as semantic clustering and recall consistency and have suggested that ecstasy related deficits may be due to combination of episodic memory *and* executive processes that

are supported by the medial temporal lobes and the prefrontal cortex respectively (Brown, McKone & Ward, 2010; Quednow et al., 2006). Thus, although the current meta-analysis has clearly identified that immediate memory and therefore initial encoding (Trial 1) is less impaired than verbal learning and consolidation processes (Total and Delayed recall) the difficulty with interpreting studies that use these standard measures of memory and learning is that Total and Delayed recall are outcome measures of multiple memorial processes. Although these summary measures have been used in the ecstasy and verbal memory literature to date, and are routinely calculated when using verbal list learning tasks for research or clinical practice, they do not adequately capture the more specific trial to trial differences that comprise the overall learning curve. The following chapter reports on ecstasy users' data from two list learning tasks similar to the CVLT and RAVLT, and includes a detailed deconstruction of trial by trial performance as well as the summary measures to better elucidate the effect of ecstasy consumption on memory processes.

Table 6.3

All Studies Included in the Meta-Analysis

Author & Location of Study	Measure	Ecstasy using group N	Ecstasy using group Mean (SD) estimated lifetime use	Controls
Bedi & Redman (2008) Australia	RAVLT Trial 1 RAVLT Delay	45	77.8 (89.1)	48 cannabis & polydrug users 40 alcohol users
Brown, et al. (2010) Australia – Sample 2	RAVLT Trial 1 RAVLT Total	30	394.5 (123.0)	34 drug naïve
Brown, et al. (2010); Australia – Sample 3	CVLT Total CVLT Delay	33	384 (109.0)	32 drug naïve 32 cannabis users
Curran & Verheyden (2003) UK	Buschke Trial 1 Buschke Delay	32	NA	32 polydrug
Halpern et al. (2004) USA	CVLT Trial 1 CVLT Total CVLT Delay	23	Median = 60	16
Halpern et al. (2010) USA	CVLT Trial 1 CVLT Total	52	Median = 43.5	59
Golding et al.(2007) UK	RAVLT Trial 1 RAVLT Delay	20	55.05 (56.0)	20 Ecstasy naive
Gouzoulis-Mayfrank et al. (2000) Germany	German AVLT Trial 1 German AVLT Delay	28	NA	28 drug naïve 28 cannabis users
Lamers et al. (2006) USA	RAVLT Total RAVLT Delay	11 ecstasy + cannabis	37.6 (33.6)	15 cannabis users 15 drug naïve

McCardle et al. (2004) Australia	RAVLT Trial 1 RAVLT Total RAVLT Delay	17	NA	15
Medina et al. (2005) USA	CVLT- Total	48	267 (482)	17 cannabis users
Parrot & Lasky (1998) UK	Auditory recall test – Single trial	15 regular users 15 novice users	NA	15
Reneman, Majoie et al. (2001) Netherlands	RAVLT Total RAVLT Delay	8	902 (801.2)	7 drug naïve
Reneman, Lavalaye et al. (2001) Netherlands	RAVLT Total RAVLT Delay	22	485.5 (598.0)	13
Schilt et al. (2008) Netherlands	RAVLT Total RAVLT Delay	31	327.8 (364)	38 polydrug users
Schilt et al. (2010) Netherlands	RAVLT Total RAVLT Delay	17	888.7 (678)	16 polydrug users [lifetime ecstasy <15] 20 drug naïve
Smith et al. (2006) USA	CVLT Trial 1 CVLT Total CVLT Delay	13	42.8 (57.8)	13 drug naïve
Thomasius et al. (2003) Germany	AVLT Trial 1 AVLT Total AVLT Delay	30	NA	29 polydrug users 30 drug naïve controls
Thomasius et al. (2006) Germany	AVLT Trial 1 AVLT Total	11	798.23 (609.29)	11 polydrug users 15 drug naïve

Wagner et al. (2012) Germany	AVLT Delay RAVLT Total	23	33.6 (7.2)	43 abstinent new ecstasy users
Yip & Lee (2005) Hong Kong	Chinese RAVLT Total Chinese RAVLT Delay	100	35.84 (13.21)	100 drug naïve

Chapter 7

Study 2: An Investigation of the Cognitive Processes in Multi-trial Word Recall Tasks that are impaired by Ecstasy and Poly-drug use

Previous studies that have used neuropsychological clinical tests of word list learning such as the AVLT and CVLT have obtained mixed results, although meta-analyses have identified verbal learning and memory to be the cognitive domain most affected by regular ecstasy use (Kalechstein et al., 2007; Laws & Kokkalis, 2007; Zakzanis et al., 2007; Rogers et al., 2009). The meta-analysis in the previous chapter aimed to specify which measures in word list learning are most impaired by ecstasy use, and identified a small effect of ecstasy use for Trial 1 and moderate effects of ecstasy use for Total and Delayed recall. These results suggest that regular ecstasy use may induce alterations to neural networks responsible for consolidation and retrieval processes. However, these measures are not independent of one another; Delayed recall scores are dependent on how many words were recalled during the learning trials, therefore Delayed recall is not just a measure of storage and retrieval, but consolidation as well. Thus, scores on these measures are limited in the extent to which they can inform about the specific cognitive processes inherent to list learning tasks that are most impaired by regular ecstasy use. The purpose of the present study was to extend and clarify the findings of the previous ecstasy and verbal memory literature. This was achieved using two different list learning tasks and by calculating memory measures in addition to Trial 1, Total and Delayed recall. These alternative memory measures are often reported in the memory and cognition literature and were developed to provide more detailed information on the processes used in list learning. The list learning tasks and measures are outlined in the following section, with particular emphasis on the memory processes and neural correlates they assess.

Verbal memory deficits for ecstasy users may be attributed to altered functioning of the medial temporal lobe.

The frequently reported verbal memory deficits for ecstasy users have usually been interpreted as evidence that ecstasy users have deficits in the storage and/or

retrieval of verbal information, probably arising from impaired medial-temporal functioning (Fox, Toplis, Turner & Parrott, 2001; Fox et al., 2002; Gouzoulis-Mayfrank, Thrimm, Rezk, Hensen & Daumann, 2003; Wagner, Becker, Koester, Gouzoulis-Mayfrank & Daumann (2011) and fMRI conducted during working memory tasks have demonstrated differences in hippocampal activation between ecstasy users and controls (Becker et al., 2012; Daumann et al., 2005; Jacobsen, Mencl, Pugh, Skudlarski & Krystal, 2004; Moeller et al., 2004). The hippocampus is innervated by serotonergic axons originating from the medial and dorsal raphe nuclei and serotonin is believed to be a key neurotransmitter involved in learning and memory (Hornung, 2003). Verbal memory has also been assessed during acute tryptophan depletion (ATD; a dietary manipulation which temporarily lowers serotonin levels). These studies have frequently reported fewer words recalled during ATD compared to placebo (Harrison et al., 2004; Klaassen et al., 1999; Riedel et al., 1999; Sambeth et al., 2009 and Schmitt et al., 2000) and in an fMRI study of verbal episodic memory, van der Veen and colleagues (2006) found that ATD reduced activity in the right hippocampus during encoding, but not the retrieval phase of the task. In rats, the hippocampus has displayed considerable serotonin receptor denervation after exposure to MDMA (Fischer et al., 1995; Hatzidimitriou et al., 1999) and regular ecstasy users have displayed alterations to central serotonergic markers (eg. Kish, Fitzmaurice, Chang, Furukawa & Tong, 2010; McCann et al., 2008; Reneman et al., 2001). Recently a prospective study of novice ecstasy users (less than 5 occasions of use) used fMRI and an associative memory task (matching a face with a profession) and reported that participants who had continued to use ecstasy one year from baseline testing showed decreased activation in the parahippocampal-gyrus compared to those who did not continue to use ecstasy, and this decreased activation showed a dose-response relationship with ecstasy, and not amphetamine or cannabis use, indicating an ecstasy-specific effect (Becker et al., 2013). This combination of reduced verbal memory scores, alterations in biochemical markers of serotonin for ecstasy users, coupled with impaired verbal memory performance under conditions of reduced serotonin availability and observed hippocampal differences for ecstasy users compared to non-users has supported the view that regular ecstasy use impairs verbal memory via alterations to

two important neural correlates of memory; serotonin receptors and neural networks within the hippocampus.

As discussed previously, the hippocampus, although a key structure in episodic memory, does not operate in isolation, with many memory models now incorporating a role for the prefrontal cortex in memory encoding and retrieval. The *Working with Memory* model (Moscovitch & Winocur, 1992) consists of two components. The *hippocampal/MTL component* is posited as a memory module that functions to form a long-term memory trace for attended information. This component is passive as it relies on associations and simple proximity between memory traces, rather than actively engaging in strategic encoding of stimuli. In contrast, the *frontal/strategic component* serves to implement strategies that organise input and output from the hippocampal module, monitoring memory traces for temporal and spatial context and using this information to strengthen encoding and guide retrieval. This component therefore works with the hippocampus to transform a passive process into a reflexive, goal directed activity. This model, and others like it are consistent with the neuroimaging memory literature which indicates prefrontal areas, including the ventral lateral prefrontal cortex and dorsolateral prefrontal cortex contribute to the selection of task relevant information and semantic properties of verbal items. These areas assist with encoding and consolidation in the hippocampus, and during retrieval the prefrontal cortex plays a role in memory search and selecting retrieval cues and strategies (Becker & Lim, 2003; Blumenfeld & Ranganath, 2008; Moscovitch & Winocur, 2002; Rugg & Wilding, 2000; Shimamura, 2000; Simons & Spiers, 2003; Tranel & Demasio, 2002).

The frontal component and verbal memory deficits for ecstasy consumers

Although the MTL has been the focus of ecstasy and memory research to date, there is some evidence that the frontal, strategic component is impaired by regular ecstasy use. The ability to organise to-be-remembered items requires intact executive functions such as forming associations between remote list items and planning and implementing goal directed strategies (Gershberg & Shimamura, 1995; Rocchetta & Milner, 1993). Ecstasy users' performance on the CVLT can indicate an important role for strategic processes in ecstasy related memory deficits because it is

comprised of words from four different categories, so participants can benefit from this embedded organisational strategy to facilitate recall. Medina, Shear and Corcoran (2005) administered the CVLT to a group of ecstasy users and a cannabis-only control group and reported that ecstasy users recalled fewer words on the short and long delay free recall measures, however when the demands on executive functions were reduced by providing a category cue at recall, the ecstasy group's memory performance did not differ significantly from the cannabis control group. Although the authors interpreted the reduced recall performance as indicative of a verbal memory deficit, it is equally possible that the lower scores in the free recall compared to the cued recall condition indicates that the ecstasy users' memory impairments may be secondary consequences of impaired use of strategies to optimise memory performance. Quednow and colleagues (2006) investigated this possibility and used the RAVLT to assess memory dysfunction in regular ecstasy users, cannabis users and drug naïve controls. They included two measures of the RAVLT that have been shown to be associated with frontal lobe functions; Recall Consistency and Retroactive Interference. Low recall consistency refers to recalling a word in an early trial and failing to recall the word on a subsequent trial. Retroactive interference is the detrimental effect of subsequent learning on the recall of previously learned target material (Woodard, 2006). The ecstasy users showed significantly poorer scores for Total recall, Recall Consistency, Retroactive Interference and Forgetting Rate (number of words lost between the final learning trial and the long delay) compared with cannabis users and controls. There was also a trend for ecstasy users to recall fewer words than drug naïve controls on Trial 1 (p = 0.10, d = -.65). From these results it was concluded that in addition to having impaired encoding, consolidation and retrieval, the low Recall Consistency and high Retroactive Interference indicated that ecstasy users were less likely to successfully organise target items to assist in recall and were less able to overcome interference, both skills inherent to executive functioning. Turning again to ecstasy users' performance on the CVLT, Brown, McKone and Ward (2010) found that ecstasy users were significantly impaired on Total free recall, Short and Long Delayed recall trials compared with cannabis users and drug naïve controls. Consistent with the results from Medina et al. (2005), when participants were provided with the category

name, in the cued recall condition, the ecstasy related recall deficit was again ameliorated.

Brown and colleagues' (2011) study is the only study thus far to specifically assess for strategic organisation during list learning by assessing the degree to which participants recalled words from the same semantic category consecutively. These semantic clustering scores showed ecstasy users engaged in spontaneous semantic organisation of material significantly less than the other groups. It is therefore apparent that the ecstasy group were less able to effectively self-initiate strategic organisation of the target items, thus producing lower recall and lower semantic clustering, which is consistent with a deficit in the frontal component of the Working with Memory model. Conversely, in the cued recall condition, a task that is classified as dependent more on the hippocampal component of the Working with Memory model, the ecstasy group were able to perform at an equivalent level to the control groups. These results therefore suggest frontally mediated strategic processes are contributing to the verbal memory deficits for ecstasy users, that have so often been attributed to impaired MTL functioning. Furthermore, the RAVLT results from Quednow et al., (2006) suggest this ecstasy related strategic impairment appears to be operating regardless of whether the list is semantically related or not, suggesting that other organisational strategies other than semantic clustering are impaired for ecstasy users. One such organisational strategy that is investigated in the current study is subjective organisation, which refers to the degree to which participants recall the same words together across multiple study-test trials.

Uncovering the processes contributing to low verbal memory scores for ecstasy users-the current study

To date, the majority of studies concerning ecstasy use and verbal memory have relied on the AVLT and CVLT to assess memory performance. These tests are quick to administer and provide information on new learning, immediate memory span, susceptibility to interference, semantic clustering and recognition performance (Spreen, Sherman & Strauss, 2006). They were designed to assess verbal memory performance in clinical, rather than research settings and are typically administered to people with self-reported memory deficits. As such, they may lack the sensitivity

to detect the more subtle memory deficits associated with recreational drug use (Fisk, Murphy, Montgomery & Waring, 2011). Furthermore, both tests have been shown to have severe ceiling effects for healthy controls (Davis et al., 2003; Uttl, 2005). Ceiling effects are problematic as they prevent participants from demonstrating the true extent of their abilities, thus compressing the range of scores and limiting the test's ability to detect the full range of individual differences. As illicit drug research often recruits university students who are matched on several demographic variables, including level of education and IQ, it is important that the tests administered are best placed to detect possibly small differences in verbal memory. As increasing the list length to between 18 and 24 words has been demonstrated to ameliorate ceiling effects (Davis et al., 2003; Uttl, 2005) the current study will has include two word lists, each comprised of twenty words. The word lists are based on the AVLT and the CVLT; one is comprised of semantically related items and is therefore categorical and the other is comprised of non-related items.

The difficulty with interpreting studies that use standard measures of memory and learning however is that Total and Delayed recall are outcome measures of multiple memorial processes. Although these summary measures have been used in the ecstasy and verbal memory literature to date, and are routinely calculated when using verbal list learning tasks for research or clinical practice, they are limited with regard to how much they can inform about the reasons for lower recall scores for ecstasy users. The Total recall score for example, is comprised of immediate memory span (Trial 1 performance) actively processing (*encoding*) the target items as they are presented, which then facilitates *consolidation* and *storage.* Total recall is also dependent on how well participants can retrieve the stored information during the recall test at the end of each learning trial. Furthermore, Delayed recall is not only assessing retention over time, but it is also dependent on Total recall scores, since the number of words consolidated during the learning trials is going to be reflected in how many words are recalled after the delay. Thus, there are many component cognitive processes that may underlie gross verbal learning and memory deficits for ecstasy users. The working with memory model provides a useful framework of the processes involved in verbal memory. The *hippocampal/MTL* component is modular and cue-dependent; its primary role is to automatically store consciously apprehended information, whereas the *frontal*/*strategic* component is

responsible for organising the information in a manner that will facilitate consolidation to, and retrieval from the hippocampal component. The *Working with Memory* posits that Trial 1 and Recognition performance are within the domain of the hippocampal component. As there is limited opportunity to engage in strategic elaboration on a single learning trial, and because Recognition is a cued recall test, both these measures reduce the need of the frontal component to strategise at encoding and guide retrieval. However Total and Delayed recall are comprised of processes that are supported by both the prefrontal cortex and medial temporal lobes, and as such it is useful to deconstruct performance on multi-trial tasks into acquisition, forgetting and strategic components.

Acquisition and forgetting during performance on multi-trial verbal learning tasks

Although standard summary measures of list learning have been used in the ecstasy and verbal memory literature to date, and are routinely calculated when using verbal list learning tasks for research or clinical practice, they do not adequately capture the more specific trial to trial differences that comprise the Total recall score. Performance within discrete trials or pairs of trials may include deficits in encoding and retrieval, but these components are not easily identified in the traditional global list learning measures. Although much of the ecstasy related verbal memory research has used list learning tasks, as yet little is known about how encoding, consolidation and rapid forgetting combine to produce the often reported memory deficits. Two measures that can assist in identifying the processes affecting list learning performance are Gained and Lost Access (Davis et al., 2003; Dunlosky and Salthouse, 1996) and Levels of Forgetting (Fisk & Warr, 1998; Montgomery et al., 2005).Trial by trial performance can be deconstructed into a measure that identifies words that were not recalled on the preceding trial (gained access) and a measure of losses in access from trial to trial (lost access). Standard list learning measures, such as Total recall, which has been the most frequently reported measure assessing ecstasy related deficits, cannot inform on the gain of items that happens at the expense of other items. For example, a participant may recall "leather", "cotton", "spider", "moth" on trial 3 and "leather," "cotton," "ant," "butterfly" on trial 4. In both trials, the recall score is 4, but a measure of gained and lost access informs that there is low recall not due to poor encoding/acquisition (since two new words were

recalled) but because acquisition appears to only occur via forgetting previously recalled items. In comparison, another participant may consistently recall the same words, indicating that consolidation is intact, but there is no new encoding or acquisition. Thus, gained access is a measure of inter-trial encoding and acquisition, and lost access measures inter-trial consolidation failure, which is in contrast to the longer term consolidation processes long assessed by Delayed recall and Recognition.

Another useful approach to examining verbal learning for ecstasy users is to study participant forgetting patterns using a Levels of Forgetting paradigm. A level is defined by how many times a word is consistently recalled over trials. For example, if a word is consecutively recalled twice, this would be referred to as level 2 learning, and thus if a word was consistently recalled twice, and then forgotten on the next trial, this would be referred to as level 2 forgetting. This paradigm provides information about whether participants are failing to recall words that were only previously recalled once (indicating the word has not been adequately encoded) or whether they are failing to recall words that they have previously recalled multiple times (in which case the words have probably been consolidated, but then forgotten). As participants proceed through the learning trials, recalling the same word should increase the strength of the memory trace and thus assist consolidation. If the word is successfully recalled four times, and then forgotten, this is referred to as level four forgetting and is likely a retrieval deficit. If the word is only recalled once, and then forgotten, this is referred to as forgetting at level one and is probably due to the word not yet being adequately encoded or consolidated (Salthouse, 1994; Montgomery, Fisk & Newcombe, 2005). This approach therefore allows forgetting to be assessed at different levels of learning, rather than just between two pairs of adjacent trials, as is the case for gains and losses.

Evaluating the strategic component using measures of subjective and semantic clustering

Performance on multi-trial list learning tasks also depends heavily on the use of memory strategies, and by including both a semantically-related word list and a non-related word list semantic and subjective organisation strategies can be assessed

for regular ecstasy users. The one ecstasy and memory study that has calculated the semantic clustering index for the CVLT reported poorer clustering for ecstasy users compared to cannabis and drug naïve controls (Brown, McKone & Ward, 2010). Subjective organisation has not been examined for ecstasy users as yet, however Quednow et al. (2006) inferred from ecstasy users' lower Recall Consistency scores that these were possibly the result of ecstasy users' not engaging in subjective clustering of items on the RAVLT. Unlike semantic clustering, which is assessed for categorical lists in which the organisational strategy is embedded, subjective organisation is dependent on participants forming their own associations between words, based on their prior knowledge of the words. By calculating both semantic and subjective clustering, the current study can better inform as to whether strategic processes associated with frontal functioning are contributing to the reported poor verbal memory scores for ecstasy consumers.

In addition to acquisition, forgetting and organisational strategies, processing speed and short term storage capacity are processes that can influence a variety of memory tasks (eg. Conway et al., 2002; Salthouse & Babcock, 1991; Unsworth & Engle, 2005) and it is possible that ecstasy related verbal memory deficits could be accounted for by differences within these domains. The current study includes a processing speed and word span task to assess whether these processes are contributing to verbal memory deficits for ecstasy users.

Residual cognitive effects of cannabis

Regular consumers of ecstasy usually report using other psychoactive substances, most commonly alcohol, cannabis and amphetamines (Gouzoulis-Mayfrank & Daumann, 2006) thus in early studies that compared the cognitive performance of ecstasy users with drug naïve controls, it was difficult to attribute differences in performance solely to the use of ecstasy. When poly-substance use was taken into account statistically, some early studies found that memory deficits among poly-drug users were more related to the amount of cannabis, rather than ecstasy that they had consumed (Croft, Mackay, Mills & Gruzelier, 2001; Dafters, Hoshi & Talbot, 2004; Rogers, 2001; Simon & Mattick, 2001). Amongst frequent (at least monthly) ecstasy users in Australia in 2011, 31% had also used cannabis in the

previous month, and 85% had used cannabis in the preceding 6 months (Sindicich & Burns, 2011). A high percentage (49%) of these 'regular' ecstasy users used cannabis and ecstasy concomitantly, and 58% reported using cannabis when coming down from a heavy session of ecstasy. These high rates of concomitant ecstasy and cannabis use are not always adequately controlled for in the ecstasy literature (Curran, 2000; Parrot, 2006). These findings indicate that a consideration of the potentially confounding effect of cannabis is warranted when testing an ecstasy using population.

Under acute conditions, cannabis use has been shown to impair performance across various cognitive domains (Crean, Crane & Mason, 2011; Martin-Santos et al., 2010; Ranganathan & DeSouza, 2006; Solowij et al., 2008). The more pertinent questions for researchers examining the long term cognitive effects associated with either cannabis or ecstasy use are; do residual cognitive deficits associated with cannabis use exist, and how long do these last? Pope et al. (2001) addressed these questions by recruiting participants who had smoked cannabis a minimum of 5000 times in their lifetime and who currently smoked daily (n = 63), a group who had previously smoked at least 5000 times but whom had smoked less than twelve times in the preceding three months (n = 45) and a group of control participants who had lifetime use of less than 50 occasions (n = 72). All participants completed a 28 day "washout" from cannabis use which was confirmed by urine analysis. A neuropsychological test battery was administered on days 0, 1, 7 and 28 to assess general intellectual function, abstraction ability, sustained attention, verbal fluency and verbal and visuospatial learning. The Bushke Selective Reminding Test provided the verbal learning measure. This is a multi-trial list learning task similar to the RAVLT, however after Trial 1, only the words that were not previously recalled by the participant are presented. The Bushke measures rate of learning across trials and also purports to differentiate between short and long term memory processes by measuring recall of items that were not presented on a given trial (Strauss, Sherman & Spreen, 2006). For verbal learning, current cannabis users recalled significantly fewer words in total and after a 30 minute delay than former users and controls on days 0, 1 and 7 and the size of these deficits were associated with higher urinary concentrations of THC at study entry. On day 28 however, there were no significant

differences in verbal learning between groups once differences in Verbal IQ were controlled for.

Pope and colleagues (2003) later reanalysed the data by investigating the relationship between age of onset of cannabis use and neuropsychological performance. The sample was divided into three groups; an early onset group (people who began using cannabis prior to age 17) a late onset group (people who began to use at age 17 or later) and a control group. There were no significant differences between late onset users and controls on the Total and Delayed recall measures of the Bushke, however the early onset group performed significantly worse on these measures, although this effect ceased to be significant once differences in Verbal IQ were controlled for.

Grant et al. (2003) conducted a meta-analysis comprising 15 studies that examined the non-acute (residual) effects of cannabis on cognition. There were a total of 1188 participants in the study, 704 of who were regular cannabis users, 484 cannabis free controls. Participants' history of cannabis use generally involved smoking cannabis multiple times per week for a duration of use ranging from two years to decades. The average length of abstinence prior to cognitive testing ranged between 24 hours to several months. The meta-analysis obtained effect sizes between cannabis users and controls across eight cognitive domains; reaction time, attention, verbal/language, abstraction/executive, perceptual/motor, motor, verbal learning, and forgetting. A global neuro-cognitve effect size was also calculated. The largest effect sizes were obtained for verbal learning (d = 0.21, 99% *CI*: -0.39, 0.02) and forgetting (d = 0.27, 99% *CI*: 0.49, 0.04). The effect size remained at a small magnitude when all 15 studies were used to calculate the global effect size (d = 0.16, 99% *CI:* 0.29, 0.03). These results are largely consistent with Pope et al., (2001) and may be due in part to symptoms of cannabis withdrawal (Haney, Ward, Comer, Foltin & Fischman, 1999; Kouri & Pope, 2000). Given the small magnitude of the effects for verbal learning and forgetting (failure to recall or recognise verbal items from list learning tasks) Grant et al. concluded that the residual impact on daily functioning for cannabis users was probably minimal, particularly since many of the participants were long term, relatively heavy users and therefore demonstrated larger deficits than might be apparent in moderate users.

Several studies have used verbal learning tasks to assess verbal episodic memory performance in cannabis users (Bolla, Brown, Eldreth, Tate & Cadet., 2002; Block, O'Leary & Hichwa, 2002; Croft, Mackay, Mills & Gruzelier, 2001; Fletcher, Page & Francis, 1996; Harvey, Sellman, Porter & Frampton, 2007; Medina et al., 2007; Pope and Yurgelun-Todd, 1996; Pope et al., 2001; Pope et al., 2002; Quednow et al., 2006; Simon & Mattick, 2002; Solowij, Stephans & Rothman, 2002; Wadsworth et al., 2006; Wagner, Becker, Gouzoulis-Mayfrank & Daumann, 2010). Deficits for cannabis users compared with drug naïve controls have been reported for Trial 1 (Medina et al., 2007), Total recall (Croft et al., 2001; Battisti et al., 2010; Solowij et al., 2002; Wagner et al., 2010), and Delayed recall (Wadsworth et al., 2006) of verbal list learning tasks, although these results have not been consistent across all studies, with several reports of no significant differences between cannabis users and controls (Medina et al., 2007; Simon & Mattick, 2002; Pope & Yurgelum-Todd, 1996; Quednow et al., 2006) . Solowij and Battisti (2008) suggest that the inconsistent findings are due to variations in age of cannabis use onset and frequency and duration of use, the latter of which has recently been identified as being most predictive of lower total recall scores for cannabis users (Wagner et al., 2010). Nevertheless, there is sufficient evidence to indicate that cannabis related deficits on verbal list learning tasks persist after the period of acute intoxication, although the magnitude of these effects are considerably smaller than those reported for ecstasy use.

The interactive effects of THC and MDMA

Ecstasy and cannabis are frequently used together, and their combined effects may be interactive rather than additive (Croft et al., 2001; Fisk, Montgomery, Wareing & Murphy, 2006; Parrot, Gouzouikis-Mayfrank, Riodgers & Solowij, 2004). Furthermore, their acute effects have opposing effect on oxidative stress. In contrast to MDMA, which has been shown to produce a hyperthermic response in humans (Ciohen & Cocolres, 1997; Freedman et al., 2005; Kolbrich et al., 2008; Vollenweider et al., 1998) THC has hypothermic properties in rats (Morley, 2004; Hampson et al., 1998; Sarne & Mechoulam, 2005) and monkeys (Taffe, 2012). The co-administration of MDMA with THC has been shown to prevent MDMA induced hyperthermia in rats and monkeys, and to partially prevent the reduction of

serotenergic markers in the rat brain (Morley, 2004). These findings have contributed to the theory that cannabis may offer some neuro-protective effect in recreational ecstasy users (Parrot, 2006). In human participants, co-administration of THC and MDMA delayed the onset of MDMA- induced increase in body temperature, however it also prolonged the duration of elevated temperature (Dumont et al., 2009). Acute co-administration of THC and MDMA has also been demonstrated to reduce the lower 2 alpha band of EEG oscillations, which is believed to reflect general attentional processes (Lansbergen et al., 2011). With regard to verbal learning and memory, Fisk, Montgomery, Wareing and Murphy (2006) investigated the interactive effects of cannabis and ecstasy use using a paired associates task. They compared ecstasy users who usually consumed ecstasy and cannabis concomitantly with ecstasy-only users and drug naïve controls, and reported no significant differences between the ecstasy-plus-cannabis and ecstasy only users on any measures of verbal paired associates, and a tendency for the ecstasy-plus-cannabis users to perform worse than non-users on initial learning and rate of forgetting. At present then, there is little evidence to support the view that concomitant cannabis use may protect against cognitive deficits associated with ecstasy use, however to date, published studies involving the co-administration of cannabis and MDMA in human participants are scarce, leaving the neuro-protective hypothesis under-investigated at this time. In summary, when taking into account the high proportion of ecstasy users who also smoke cannabis, combined with the individual effects of cannabis and ecstasy on verbal memory outcomes and the possibility that cannabis has neuro-protective properties, cannabis use will be controlled for in the present study by including it as an independent variable.

Examining the effects of poly-substance use on verbal memory

There has been much conjecture in the ecstasy and memory literature about whether ecstasy use is responsible for the observed cognitive deficits in regular users, or whether it is the effects of poly-substance use that is impairing cognition. Much of this conjecture was based on the methodological difficulties associated with cross sectional designs, including pre-morbid differences in estimated IQ, education, and most importantly, poly-substance use. This debate was revisited after Halpern and colleagues (2010) conducted a study of the cognitive effects of ecstasy use by

recruiting participants who had either used or not used ecstasy and who all had very minimal exposure to other illicit drugs and alcohol. They used the CVLT to assess verbal memory performance, and reported no significant differences between the poly-drug –plus- ecstasy group and the poly-drug only group on Immediate (g = -.04) and Total recall (g = -.12) measures of the CVLT. Halpern and colleagues concluded that by rigorously controlling for the potentially confounding effects of other drug use in their sample they failed to demonstrate marked residual cognitive deficits in ecstasy users and this might indicate that ecstasy use by itself does not produce lasting residual neurotoxicity. To specifically investigate the possibility that poly-drug-plus-ecstasy use can account for verbal memory deficits on a list learning task, the current study includes four groups; participants who use ecstasy with very minimal exposure to other illicit drugs, participants who use ecstasy and cannabis with very limited exposure to other illicit drugs, participants who use cannabis only and a drug naïve control group. Controlling for poly-substance use in the design of the study will therefore allow the effects of poly-substance use on verbal memory to be adequately assessed.

Aims of the present study

To summarise, the present study aims to investigate the underlying multiple cognitive processes inherent to performance on list learning tasks that may account for verbal memory deficits associated with ecstasy use. To achieve this, organisational strategies, encoding, consolidation and retrieval and inter-trial forgetting patterns will be examined, and the choice of these measures were informed by the Working with Memory model to elucidate the respective roles of the prefrontal and medial temporal cortices in list learning performance for ecstasy users. In light of recent evidence that suggests it may be poly-substance rather than ecstasy use that is responsible for cognitive deficits reported for ecstasy users, the present study will specifically investigate this by comparing an Ecstasy-only and Ecstasy-plus cannabis (poly-substance group) with a Cannabis-only and a Drug naïve group, thus extricating the contributions each drug makes to cognitive functioning, as well as their interactive effects.

Based on the results of the previous meta-analysis, it was expected that people who had used ecstasy would demonstrate poorer performance on Trial 1, Total and Delayed recall of both the related and unrelated word list tasks. Alternatively, if an Ecstasy x Cannabis interaction is observed, this will be examined to ascertain whether poly-substance use is impacting on the memory measures. If ecstasy users display lower levels of semantic or subjective organisation relative to cannabis users and controls, this would indicate that poor memory scores are partly due to impaired frontal functioning. Alternatively, if ecstasy users have poorer recall but are comparable to non-ecstasy users on the organisational measures, this would suggest the memory deficits are more associated with memory-pure processes, such as encoding, consolidation and retrieval deficits. Further, if ecstasy users do differ from non-ecstasy users on Total and Delayed recall, consideration of gained and lost access and levels of forgetting can inform as to whether this is likely due to poor encoding/acquisition (low gained access) or consolidation (high lost access) or a combination of both.

Method

Participants

Participants were recruited through advertisements distributed throughout the University of Tasmania and through peer-referral. Prospective participants were asked to contact the researcher via telephone, and were screened for eligibility. Seventy-nine people were recruited for the study and allocated to one of four groups based on their drug-use history: an ecstasy-only group (minimum of 10 occasions of ecstasy use; at least six in the past six months, minimal use of other illicit drugs), a cannabis-only group (minimum of 10 occasions of cannabis use; at least six in the past six months, minimal use of other illicit drugs), an ecstasy-plus-cannabis group (as for the ecstasy-only and cannabis-only groups), or a drug-naive control group (a maximum of five lifetime occasions of illicit drug use; none in the last six months). Subsequent examination of demographic and drug-use information resulted in ten participants being excluded. Eligible participants were over 18 years of age, of normal or corrected-to normal vision, spoke English as their first language, were free of pre-existing neurological or psychological disorders, had no psychotropic medication use in the month prior to testing, and no other notable history of other illicit drug use (exceeding five lifetime doses).

Participants were asked to abstain from ecstasy or cannabis use for three days prior to their testing session in order to avoid the residual acute or sub-acute effects of these substances. The sub-acute effects of ecstasy intoxication include low mood and/or poor concentration and are believed to subside within 72 hours (Parrot & Lasky, 1998). The acute psychotropic effects of cannabis are suggested to abate after three hours (Grothenhermen, 2003). Additional exclusion criteria therefore included reported ecstasy use in the seven days prior to testing, and reported cannabis use in the 24 hours prior to testing.

Materials

A demographic questionnaire was included to ensure equality between the groups. Assessment included sex, age, education level, recent caffeine and nicotine intake, general medical information (neurological and psychiatric conditions and any prescribed medications), and the Karolinska Sleepiness Scale (Guilleminault &

Brooks, 2001) to assess current level of fatigue. The Kessler Psychological Distress Scale (K10; Kessler & Mroczek, 1992, 1994) was administered to assess levels of non-specific psychological distress, and has been demonstrated to possess adequate reliability and validity (Andrews & Slade, 2001). The Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, de la Fuente, & Grant, 1993), which has been shown to be a reliable and valid instrument for assessing harmful alcohol consumption (Dawe, Loxton, Hides, Kavanagh, & Mattick, 2002) was included to assess problematic alcohol use in the preceding 12 months. Lifetime Drug-use Interview procedures (LDU; Czermak et al., 2005) were employed to assess the extent of participants' lifetime drug exposure. Although it would have been preferable to have conducted urinary tests to verify self- reported drug use, the self-report method of obtaining drug use history for research purposes has been shown to have sufficient reliability and validity (Darke, 1998; Harrison, Martin, Enev, & Harrington, 2007), and the LDU has been demonstrated to have high test-retest reliability (Czermak et al.). Additionally, the Q-score index (Q= amount consumed on last two occasions/days since two most recent occasions of use) from the Opiate Treatment Index (OTI; Darke, Ward, Hall, Heather, & Wodak, 1991) was measured to determine a measure of recent ecstasy and cannabis use that reflects dose and frequency. The OTI has been demonstrated to be a reliable and valid assessment tool (Darke, Ward, Hall, Heather, & Wodak, 1991.).

The WTAR (Wechsler, 2001) is a reading recognition test comprised of 50 words with irregular pronunciation (e.g. gnome). It has high validity and reliability (Ginsberg, Risser, Purisch, Hamilton, & Burns, 2003), and is a strong predictor of general intelligence (Green et al., 2008). It was administered to assess between group differences in general intellectual functioning, with raw scores converted to age-normed United Kingdom standard scores.

Non-related list stimuli

A list of 40 words selected from the Medical Research Council Psycholinguistic Database, version 2.0 (Colthart, 1981, available online at http://www.psy.uwa.edu.au/mrcdatabase/uwa_mrc.htm) comprised the non-related list and Recognition task stimuli. Like the CVLT and RAVLT, all words were concrete nouns, with a mean concreteness rating of 569 (range: 471-634). The words had a

mean word frequency of 7 per million (range: 1-19 million) according to the Kucera and Francis norms (1967). The low level of word frequency was chosen to increase the difficulty of recall, as free recall is generally found to be poorer for low frequency words (Balota & Chumbley, 1984; Jescheniak & Levelt, 1994). Due to the tendency for shorter words to be more easily recalled than longer words (eg. Baddely, Thomson & Buchanan, 1975) all words were between 5 and 8 letters long (mode: 6). Of these words, twenty were randomly selected as the list stimuli and twenty comprised part of the Recognition test. The list learning tasks were created and run in Inquisit (Version 2.0). Stimuli are presented in Appendix A.

Related list stimuli

The related list consisted of 20 concrete nouns, comprising four words from each of five semantic categories; geographical features, insects, types of fabric, musical instruments, vegetables. The categories and exemplars were chosen from an updated version (Van Overschelde, Rawson & Dunlosky, 2004) of the Battig and Montague (1969) norms. The words had a mean word frequency of 5 per million (range: 1-12 million) and a mean concreteness rating of 599 (range: 574-633). All words were between 6 and 9 letters long (mode: 6). Stimuli are presented in Appendix A.

List learning and recall procedure

Task administration was computerised, using an IBM compatible computer with a 40 centimetre screen. Before the learning phase of the task, participants were informed they were going to be presented with a list of words, and that the aim of the task was to try and remember all the words. Following instruction, words were presented one at a time, in lowercase, black, Arial, 36 point font in the middle of the screen against a white background. Each word remained on the screen for a duration of 2000 milliseconds, with an inter-stimulus interval of 250 milliseconds. The order of presentation was randomised across each learning trial. At the end of the learning phase, the recall phase began, in which participants were asked to recall all the words they could remember, in any order they wished. For the unrelated list, this procedure was repeated for eight learning and recall phases and for the related list there were only five learning and recall phases. Reducing the number of trials for the related list

was based on the pilot study in which participants often reached asymptote at Trial 5, and due to reports that the overall testing session was fatiguing. Due to the long and effortful nature of the task, after the sixth presentation of the unrelated list, participants were informed they had two more learning trials until completion.

Delayed recall and Recognition procedure

Delayed recall and recognition were conducted for the unrelated list only. Thirty minutes after the unrelated list task, during which participants performed a visual tracking task for a separate study and a letter comparison task (described below) participants were reminded of the list of words they had learnt and were asked to recall all the words from that list that they could remember. Following Delayed recall, the Recognition task commenced. This consisted of the twenty words from the non-related list and twenty additional items. Words were presented one at a time on the screen, in a randomised order for each participant. Participants were informed they were going to be presented with another list of words, and that some were from the original list, and some were not. They were instructed to respond YES if the word was on the original list and NO if it was not, by pressing the corresponding keys on computer the keyboard. Words remained onscreen until the participant had responded, after which it was replaced by the next word. Recognition accuracy was recorded by the program.

Letter comparison task stimuli and procedure

The letter comparison task was based on Salthouse's (1994) version. Stimuli for the letter comparison task consisted of 90 pairs of letter strings consisting of three, six or nine letters. There were 30 pairs for each string length. Letters were presented in black, 26 point, Arial font in the middle of a white screen. One half of the pairs were different because of a change in the position or identity of one of the letters in one member of the pair.

Participants were instructed to decide if the two rows of letters were the same or different, as quickly and as accurately as possible. The pairs of letter strings were presented in 3 blocks of 30, and each block contained strings of the same length. The first block consisted of pairs of three letters, the next six and the final consisted of nine. Trials within each block were randomised for each participant. Participant

responses were made by pressing the corresponding button on the computer keyboard and accuracy and reaction times were recorded by the Inquisit program.

Word span task stimuli and procedure

The word span task (Salthouse & Babcock, 1991) stimuli consisted of 105 mono-syllabic nouns, all 4-5 letters in length. For the study phase, participants were instructed that the aim of the task was to remember the words in the same order as they were presented. Words were presented one at a time in black, 36 point Arial font in the middle of the screen, for a duration of 1000 milliseconds, with an inter-stimulus interval of 250 milliseconds. The number of words per trial varied from 2 to 8, and trial length started at 2 and increased by one word every three trials, thus there were three trials of the same length per block. At the end of each trial, participants were asked to recall the words in the same order of presentation. The task ceased when a participant failed to correctly recall all three trials. Word span was defined as the highest series length recalled in the correct order.

Design and data analysis

A 2 [Ecstasy use: present, absent] × 2 [Cannabis use: present, absent] factorial design was used, with ecstasy and cannabis use as the between subjects factors and various measures of memory as the dependent variables. The standard memory measures are summarised in Table 7.1 below. For the inter-trial measures, the gained access score was defined as the number of words recalled on trial $n + 1$ that were not recalled on n, and lost access was defined as the total specific words recalled on trial n that were not recalled on trial $n + 1$. For levels of forgetting, the incidence of forgetting was measured at different levels of learning (a level is defined as the number of times a specific word had been consecutively recalled.) For example, forgetting a word that had been previously recalled once would indicate forgetting at level 1, forgetting a word that was recalled twice would be forgetting at level 2, and so on. The frequency of forgetting at each level was summed over all trials which provided 7 possible levels of forgetting.

Table 7.1

*Descriptions and calculations for Main Memory Measures for List Learning Task*s

Dependent Variable	Calculation	Description
Immediate memory Trial 1	Number of words recalled after first list presentation	Short term memory for a single trial
Trials 1…8	Number of words recalled on each learning trial	Learning- provides measure of the extent to which recall improves as the number of learning trials increase
Total recalled	Sum of all words recalled from trials 1 – 8	Consolidation, learning and retrieval
Learning over Trials	Sum of words remembered across trials 1 – 8, corrected for immediate word span (Total recall – 8 x Trial 1 score).	Measure of learning, corrected for differences on immediate memory span
Measures of organisation		
Bidirectional paired frequency[+] (Tulving, 1962)	PF = O(IRT2) – E(IRT2) = O(IRT2) – 2c (c - 1) /hk	The extent to which a subjective organizational strategy is used to assist recall on a learning trial
List based clustering index[#] (Stricker, Brown, Wixted, Baldo & Delis, 2002)	$OBS - \frac{[(r-1)(m-1)]}{Nl-1}$	The extent to which a semantic clustering strategy is used to assist word recall on a learning trial
Measures after delay[^]		
Delay	Number of words recalled after 30 minute delay	Long term consolidation and retrieval
Forgetting rate	Number of words recalled at Trial 8 minus number of words recalled delay	Number of items forgotten during delay, strength of consolidation, retrieval
Recognition	Number of correctly identified targets on 40 item recognition task	Long term memory retention/storage.

Table notes from previous page: [+] Bi-directional pair frequency scores refers to the number of times a pair of words appear together over two consecutive trials, regardless of their order of output. O(ITR2) represents the number of pairs of items recalled on Trials *t* and *t*+1 in adjacent output positions in either of two possible orders, E(IRT2) represents the expected number of pairs of items, where *c* is the number of common items recalled on both Trials t and *t*+1, *h* is the number of items recalled on Trial *t*, and *k* is the number of items recalled on Trial *t*+1 (Tulving, 1962).
[#] Related list only, where OBS = the number of observed clusters on a trial (a cluster is defined as two words from the same category being consecutively recalled). r = the number of correctly words on a trial, m = number of semantic categories and NL = number of words on the list (Stricker, Brown, Wixted, Baldo & Delis, 2002).
[^] Unrelated list only.

Analyses were conducted using IBM SPSS 20. Demographic and drug use history variables were analysed using one-way ANOVAs and chi-square approaches, with significant main effects followed up with Tukey post-hoc tests. Memory related measures were analysed using between groups or mixed ANOVA. Where repeated measures ANOVAs were used Greenhouse-Geisser adjustments were applied to correct for violations of assumptions of sphericity. Planned one-way ANOVAs were performed and Bonferoni adjusted as required and significant main effects were followed up with Bonferoni adjusted pairwise comparisons. Effect size measures (Hedge's *g*) were calculated for drug related main effects and were classified according to Cohen's (1969) recommendation; $g = 0.20 – 0.49$; a small effect, $g = 0.50 – 0.79$; medium effect and $g > 0.80$; large effect. Dose dependence was ascertained using bivariate correlations between the memory variables of interest and drug use measures. If dose dependence was found for drugs other than ecstasy ANCOVA was conducted to determine whether the ecstasy-related effects remained after the influence of other drug use was taken into account. Where the other drugs were not a significant covariate the unadjusted analyses are retained.

Results

Demographic variables

To reduce the possibility of confounds to the memory analyses, groups were compared on a number of demographic variables (Table 7.2). The groups did not differ significantly on the demographic variables of sex, age, level of psychological distress as measured by the K10, fatigue at time of testing as measured by the Karolinska

Sleepiness Scale, caffeine and nicotine intake on the day of testing, and general intellectual functioning as measured by the WTAR. Groups did significantly differ on commencement of tertiary study, the ecstasy plus cannabis group had fewer participants who had commenced tertiary education compared with the ecstasy only, cannabis only and drug naïve groups, although the ecstasy only and cannabis only groups had more participants who had commenced tertiary education compared with the drug naïve group. In summary, with the exception of commencement of tertiary education, the groups did not differ on any demographic variables, indicating that any differences between groups on memory performance would not be due to age, sex, mental health status, fatigue, acute effects of caffeine and nicotine or general intellectual functioning.

Drug-use variables

The experimental groups were compared on days of use in the past six months and total occasions of use for various illicit drugs. For ecstasy use, additional comparisons for the most pills used on one occasion and estimated lifetime total dose was also assessed to ascertain if a dose-response relationship may be present (Bolla, McCann, & Ricaurte, 1998). For ecstasy and cannabis use, a measure of recent consumption (frequency and amount used on the last three occasions, measured using the OTI Q-score method: dividing the amount consumed on the previous two occasions by the time period between them) was also obtained (see Tables 7.2 – 7.8).

Table 7.2

Group Percentages, Means, Standard Deviations and ANOVA Results for Demographic Variables

Control variable	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Sex (% female)	52.4%	40%	56.3%	47.1%	$\chi^2 = 0.94$	.81
Level of Education (% commenced tertiary)	57.1	66.7	68.8	47.1	$\chi^2 = 25.29$	**.001**
Age (years)	25.3(7.9)	24.2 (5.2)	22.9 (2.2)	21.2 (2.3)	2.1	.11
Level of Psychological Distress (K10)	19.2 (5.6)	17.1 (5.7)	17.8 (5.6)	19.8 (7.1)	0.6	.56
Fatigue (Karolinska Sleepiness Scale)	4.00 (1.4)	3.6 (1.50)	3.75 (1.39)	4.6 (1.7)	1.3	.27
Caffeine Intake	1.07 (1.4)	0.6 (0.6	0.81 (1.2)	0.4 (0.7)	1.2	.31
Nicotine Intake	0.14 (0.4)	0.6 (1.7)	0.73 (1.5)	0.4 (1.0)	0.8	.49
General Intellectual Functioning (WTAR)	109.6 (11.1)	112.8 (11.2)	109.5 (8.7)	107.6 (12.3)	0.6	.59

Table 7.3

Group Means, (Standard Deviations) and ANOVA Results for Ecstasy

Drug	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Ecstasy	$n^+ = 0$	$n^+ = 8$	$n^+ = 16$	$n^+ = 17$		
Days used in past 6 months	0.00	0.00	13.69 (7.09)	16.12 (11.42)		
Period of abstinence (days)	n/a	1124.9 (1623.0)	25.44 (21.65)	16.06 (12.24)		
Number of pills used per session	n/a	n/a	1.75 (0.68)	2.76 (1.97)		
Total occasions of use	n/a	1.47 (2.03)	68.38 (60.98)	64.65 (39.18)		
Lifetime total dose	n/a	1.86 (3.22)	130 (164.12)	162.41 (153.08		
Extent of recent use (OTI Ecstasy)	n/a	n/a	0.19 (0.18)	0.26 (0.40)		

Note: $^+$ number within group with lifetime use in the particular drug class (however total *n* was used in the generation of means and *SD*s). *F* and *p* values refer to one-way ANOVAs comparing the 4 groups

Table 7.4

Group Means, (Standard Deviations) and ANOVA Results for Cannabis

Drug	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Cannabis	$n^+ = 8$	$n^+ = 15$	$n^+ = 14$	$n^+ = 17$		
Days used in past 6 months	0.00	39.07 (34.78)	0.38 (.71)	49.76 (36.79)		
Period of abstinence (days)	2247.78 (1598.71)	11.67 (16.35)	460.43 (451)	6.71 (4.98)	25.92	**<.001** EC, C, E < N
Total occasions of use	1.43 (2.08)	1372.07 (1723.28)	144.06 (406.73)	648.29 (1282.09)	5.85	**.001** C, EC, > E, N
Extent of recent use (OTI Cannabis)	n/a	1.80 (2.06)	n/a	1.27 (1.63)		
CUDIT	n/a	21.20 (11.87)	n/a	25.29 (18.35)		
Usually use cannabis with ecstasy$^{\Theta}$ (% yes)	n/a	0%	0%	82.4%		

Note: $^+$ number within group with lifetime use in the particular drug class (however total *n* was used in the generation of means and *SD*s). *F* and *p* values refer to one-way ANOVAs comparing the 4 groups

Table 7.5

Group Means, Standard Deviations and ANOVA Results for Stimulants

Drug	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Amphetamines	$n^+ = 1$	$n^+ = 4$	$n^+ = 14$	$n^+ = 14$		
Days used in past 6 months	0.00	0.00	0.69 (0.87)	1.12 (1.40)		
Total occasions of use	0.14	0.60 (1.35)	5.06 (6.19)	4.71 (5.56)	7.03	**<.001** E, EC > C, N
Pharmaceutical Stimulants	$n^+ = 0$	$n^+ = 2$	$n^+ = 3$	$n^+ = 6$		
Days used in past 6 months	0.00	0.13 (0.51)	0.00	.35 (0.99)		
Total occasions of use	0.00	0.67 (2.09)	2.56 (7.72)	3.65 (9.76)	1.33	.271
Cocaine	$n^+ = 0$	$n^+ = 6$	$n^+ = 9$	$n^+ = 7$		
Days used in past 6 months	0.00	0.27	0.50 (0.89)	0.29 (0.58)	1.56	.206
Total occasions of use	0.00	3.20 (10.41)	3.38 (5.26)	2.65 (6.29)	1.22	.308

Note: $^+$ number within group with lifetime use in the particular drug class (however total *n* was used in the generation of means and *SD*s). *F* and *p* values refer to one-way ANOVAs comparing the 4 groups

Table 7.6

Group Means, Standard Deviations and ANOVA Results for Inhalants, LSD, Mushrooms and Ketamine.

Drug	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Inhalants	$n^+ = 1$	$n^+ = 5$	$n^+ = 8$	$n^+ = 14$		
Days used in past 6 months	0.00	0.00	1.38 (4.99)	5.24 (7.86)		
Total occasions of use	0.05 (0.21)	3.40 (7.99)	5.25 (10.71)	32.06 (71.45)	2.92	**.041** EC > N
LSD	$n^+ = 0$	$n^+ = 6$	$n^+ = 6$	$n^+ = 11$		
Days used in past 6 months	0.00	0.40 (1.54)	0.81 (2.10)	1.59 (1.77)		
Total occasions of use	0.0	4.07 (7.32)	2.25 (4.55)	3.35 (5.25)		
Mushrooms	$n^+ = 3$	$n^+ = 9$	$n^+ = 6$	$n^+ = 9$		
Days used in past 6 months	0.00	0.40 (0.82)	0.25 (0.77)	1.94 (4.16)		
Total occasions of use	0.14 (0.35)	2.67 (5.08)	1.25 (2.04)	7.24 (11.99)	4.13	**.010** EC > C, E, N
Ketamine	$n^+ = 0$	$n^+ = 0$	$n^+ = 3$	$n^+ = 3$		
Total occasions of use	0.00	0.00	0.56 (1.75)	0.41 (1.22)		

Notes: $^+$ number within group with lifetime use in the particular drug class (however total *n* was used in the generation of means and *SD*s). No ketamine use in previous 6 months

Table 7.7

Group Means, Standard Deviations and ANOVA Results for Tobacco and Alcohol

Drug	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Tobacco	$n^+ = 10$	$n^+ = 11$	$n^+ = 14$	$n^+ = 13$		
Days used in past 6 months	20.67 (54.11)	61.93 (77.88)	48.88 (69.80)	76.35 (81.76)	2.14	.104
Total occasions of use	311.76 (699.53)	1023 (1714)	777.13 (1119.35)	641.35 (976.29)	1.20	.313
Alcohol	$n^+ = 20$	$n^+ = 15$	$n^+ = 16$	$n^+ = 17$		
Days used in past 6 months	32.14 (33.14)	50.40 (35.15)	34.94 (26.34)	46.47 (28.64)	1.39	.253
Total occasions of use	611.48 (690.60)	1150.27 (1743.38)	612.56 (444.12)	560.18 (523.54)	1.32	.273
Problematic Alcohol Use (AUDIT)	5.24 (4.34)	11.93 (5.66)	11.13 (7.37)	15.75 (6.48)	9.93	**.001** E, EC, C > N

Note: $^+$ number within group with lifetime use in the particular drug class (however total *n* was used in the generation of means and *SD*s). *F* and *p* values refer to one-way ANOVAs comparing the 4 groups

Table 7.8

Group Means, (Standard Deviations) and ANOVA Results for Antidepressants, Benzodiazepines and Other Opiates

Drug	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Antidepressants	$n^+ = 1$	$n^+ = 1$	$n^+ = 4$	$n^+ = 1$		
Days used in past 6 months $^{\Delta}$	0.0	0.0	2.5 (0.10)	0.0		
Total occasions of use	2.8 (13.09)	12.1 (46.99)	36.8 (98.74)	0.1 (0.24)	1.68	.179
Benzodiazepines	$n^+ = 5$	$n^+ = 3$	$n^+ = 9$	$n^+ = 10$		
Days used in past 6 months $^{\Delta}$	0.0	0.4 (1.55)	0.5 (0.96)	1.6 (2.80)		
Total occasions of use	3.0 (13.05	3.3 (10.30)	9.1 (27.11)	2.1 (2.92)	0.66	.575
Other Opiates	$n^+ = 0$	$n^+ = 4$	$n^+ = 0$	$n^+ = 5$		
Total occasions of use	0.0	0.5 (1.55)	0.0	0.3 (.84)	1.26	.291

Note: $^+$ number within group with lifetime use in the particular drug class (however total *n* was used in the generation of means and *SD*s). No opiate use in previous 6 months.
F and *p* values refer to one-way ANOVAs comparing the 4 groups

Consistent with selection criteria for group allocation, there were significant differences between groups in their ecstasy and cannabis use. For ecstasy use, the Ecstasy-only and Ecstasy-plus cannabis groups did not differ significantly on the number of days they had used in the last 6 months, number of days since last used, extent of recent use, total occasions used and lifetime dose, and both the ecstasy groups were significantly higher on these variables compared with the Cannabis-only and drug naïve control groups. For cannabis use, the Cannabis-only and Ecstasy-plus cannabis groups did not significantly differ on number of days used in the past 6 months and total occasions of use, and both groups reported significantly higher frequency of use compared with the Ecstasy-only and drug naive groups. The Cannabis-only and Ecstasy-plus cannabis groups were also well matched on CUDIT and OTI scores for cannabis use.

For cocaine, pharmaceutical stimulant, ketamine, tobacco, antidepressant, alcohol and opiate use the differences between groups were non-significant. There were also no differences between groups for total occasions of LSD and benzodiazepine use. For problematic alcohol use, Tukey post hoc testing showed the ecstasy, Cannabis and Ecstasy-plus cannabis groups all scored significantly higher than the drug naïve controls.

The Ecstasy-only, Cannabis-only and Drug naïve groups were well matched for the number of days they had used amphetamines, LSD, mushrooms, inhalants and benzodiazepines in the last 6 months and for total occasions of inhalant, mushroom and amphetamine use. The Ecstasy-plus cannabis, Ecstasy-only and Cannabis-only groups did not significantly differ on past 6 month use of LSD, benzodiazepines and mushrooms, however the Ecstasy-plus cannabis group reported more use of these drugs in the past 6 months compared with the Drug naïve controls. This poly-drug group had also used more inhalants in the last six months (an average of five occasions) compared with the Cannabis-only and Drug naïve groups who reported no inhalant use in the preceding 6 months.

Mushroom use was low for all groups; the Ecstasy-plus cannabis group reported an average of 7 occasions of lifetime use compared with an average of 1

occasion of use for the Ecstasy-only group. The total occasions of amphetamine use was also higher for the ecstasy and Ecstasy-plus cannabis groups relative to the Cannabis and Drug naïve groups, although again, overall use of amphetamines was quite low, both the Ecstasy-only and Ecstasy-plus cannabis groups had used amphetamines an average of 5 times in their lifetime. Overall, due to the low frequency of drug use other than ecstasy or cannabis in this group of participants, particularly within the preceding six months, these differences are minimal.

List learning analyses- between lists

To investigate the effects of ecstasy consumption on verbal memory across the two different list types, a 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) × 2 (List; non-related, related) × 5 [Trial; 1-5] mixed ANOVA was performed. As expected, the effect of List type was significant, $F(1, 64) = 122.25$, $p < .001$, $g = 1.52$, with participants recalling significantly more words per trial on the related list ($M = 14.44$, $SD = 1.70$) compared with the unrelated list ($M = 11.81$, $SD = 1.70$). Similarly, the effect of Trial was significant, with participants recalling significantly more words as the number of learning trials increased, $F(4, 256) = 390.72$, $p < .001$.

There was a significant and large magnitude main effect of Ecstasy, $F(1, 64) = 19.8$, $p < .001$, $g = -1.07$, with ecstasy users recalling significantly fewer words than non-ecstasy users across both lists. There was no significant interaction between Ecstasy and Cannabis (F (1, 64) = 1.26, $p = .26$). The List × Ecstasy interaction was also non-significant, $F(1, 64) = 1.53$, $p = .22$, indicating that the lower recall scores for ecstasy consumers were consistent for the two list types. Figure 7.1 shows the mean number of words recalled per trial for ecstasy users compared to non-ecstasy users across both lists.

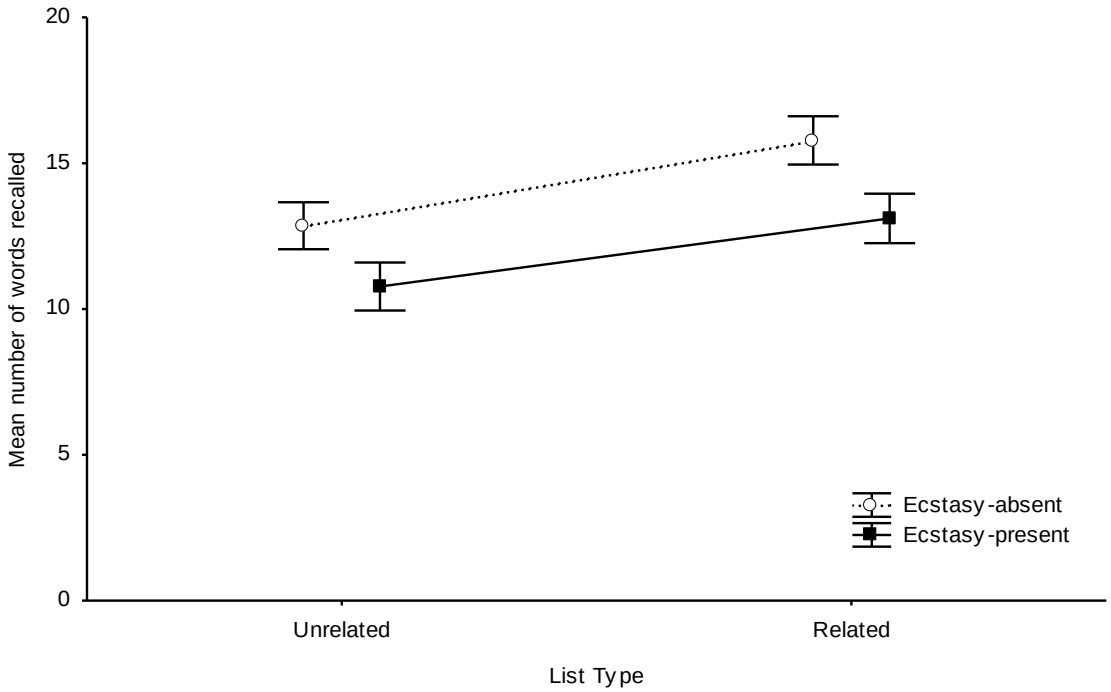

Figure 7.1 Mean number of words recalled per trial between the related and unrelated lists for ecstasy and non-ecstasy users (error bars represent standard errors.)

In contrast, the lower recall scores for ecstasy users were not consistent across all learning trials as evidenced from the significant Trial × Ecstasy interaction, $F(1, 256) = 2.93$, $p = .04$. To ascertain the nature of this interaction, a series of one way Bonferroni-adjusted ANOVAs compared the mean trial recall scores (averaged between lists) for Ecstasy-present and Ecstasy-absent groups at each trial. As shown in Figure 7.2, ecstasy users had significantly lower recall scores across all learning trials with the exception of Trial 1. Ecstasy use was not involved in any other higher order interactions, with Ecstasy × Cannabis × List × Trial, $F(4, 256) = 0.15$, $p = .95$, Ecstasy × Cannabis × List, $F(1, 64) = 0.46$, $p = .50$, Ecstasy × Cannabis × Trial ($F(1, 64) = 0.43$, $p = .70$), Ecstasy× List× Trial, $F(4, 256) = 0.16$, $p = .95$, all failing to reach statistical significance.

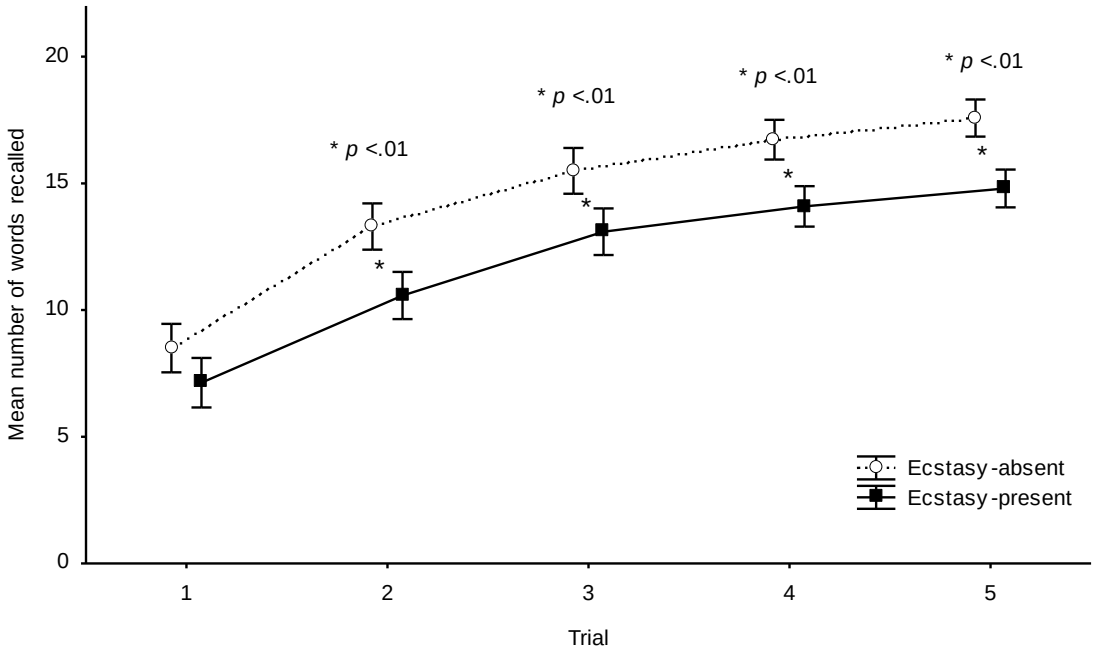

Figure 7.2. Mean number of words recalled between the unrelated and the related list for each trial as a function of the presence and absence of ecstasy use (error bars represent standard errors.)

The main effect of Cannabis was not significant, $F(1, 64) = .48$, $p = .49$, $g = -.17$, and power analysis revealed that 1090 participants would have been required to reliably (*Power* = .8) identify an effect of this magnitude as significant. The Cannabis× List, $F(1,64) = .09$, $p = .76$, Cannabis× Trial, $F(4,256) = .42$ $p = .71$, and Cannabis× List× Trial interactions, $F(4, 256) = .68$, $p = .59$, were also non-significant.

Summary

Overall, as would be anticipated, more words were correctly recalled on the semantically related compared with the unrelated list and more words were recalled as the number of learning trials increased. Importantly, ecstasy users recalled fewer words than non-ecstasy users across all bar the first trial, in which all participants recalled a similar number of items. These ecstasy related differences occurred regardless of the comorbid presence or absence of cannabis use. List type was not involved in any drug related interactions, indicating that ecstasy users performed

more poorly regardless of whether the list possessed categorical properties that could assist with recall. Given the lack of list-related interactions, the unrelated and related lists were each analysed separately to investigate several dependent variables, some of which are specific to each list type. Treating the lists separately also allows finer grained, trial by trial analyses to be conducted, to examine whether words were lost or gained from one trial to the next.

Unrelated list, traditional measures: The effects of ecstasy use on memory for unrelated words, using a list learning paradigm.

To examine the learning curve for each of the groups, the number of words recalled on the unrelated list task was first analysed using a 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) × 8 [Trial; 1-8] mixed ANOVA. As predicted, the within subjects effect of Trial was significant, $F(7,64) = 230.62$, $p < .001$ and Bonferroni-adjusted pairwise comparisons showed the number of words correctly recalled by participants overall significantly increased between trials 1 to 4, did not differ significantly between trials 4 and 5, 5 and 6, and 6 and 7, and increased significantly between trials 7 and 8.

The main effect of Ecstasy was significant and large in magnitude, $F(1, 64) = 14.87$, $p = <.001$, $g = .92$, with ecstasy users recalling significantly less words averaged across trials ($M = 12.45$, $SD = 2.18$), than non-ecstasy users ($M = 14.51$, $SD = 2.21$). This effect was independent from the effects of Cannabis as evidenced by the non-significant Ecstasy × Cannabis interaction, ($F(1, 64) = 2.01$, $p = .16$. There was no significant Ecstasy x Trial interaction ($F(4, 64) = 1.47$, $p = .20$) which indicates that ecstasy users recalled fewer words than non-users on each learning trial. As can be seen in Figure 7.3, the profiles for the ecstasy users and non-users are similar, with the only difference between the learning curves being the number of words correctly recalled for each trial.

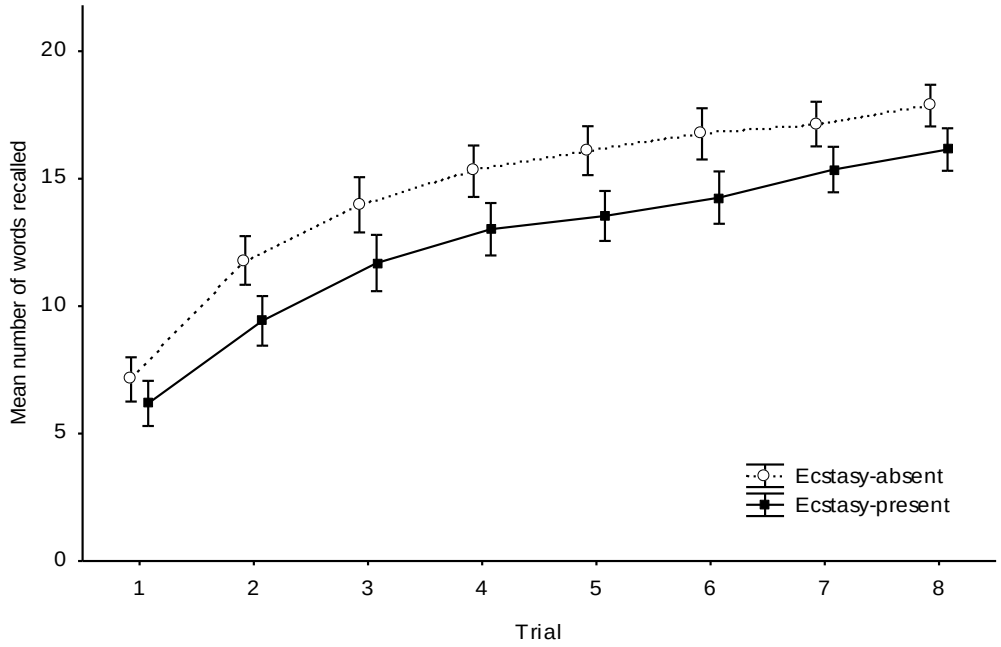

Figure 7.3. Mean number of words recalled on each trial of the unrelated list based as a function of status of ecstasy use (error bars represent standard errors.)

There was no effect of Cannabis on number of words recalled overall across trials, $F(1, 64) = .33$, $p = .56$, $g = .14$, and power analysis revealed that 1604 participants would have been required to reliably (*Power* = .8) identify an effect of this magnitude as statistically significant. The Cannabis × Trial, $F(4, 64) = .52$, $p = .75$, and Trial × Cannabis × Ecstasy, $F(4, 64) = .20$, $p = .96$, interactions were all non-significant.

Immediate memory and learning measures for the unrelated list

To further investigate immediate memory, learning variables and delayed recall, a series of 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) ANOVAs were performed with Cannabis (present, absent) and Ecstasy (present, absent) as the between subjects factors and the memory measure as the dependent variable. The results from the univariate analyses for the unrelated list are summarized in Table 7.9 below. As can be seen, there were no significant Cannabis main effects or Ecstasy × Cannabis interactions. However four significant effects of Ecstasy were found.

As shown in Table 7.9, there was no significant main effect of Ecstasy for Trial 1, although there was some indication of a small magnitude effect, g = -.36, with ecstasy users (M = 6.18, SD = 2.54) to perform more poorly than non-users (M = 7.12, SD = 2.57). As shown in Figure 7.4, for Total Recall across all learning trials, the Ecstasy users recalled significantly fewer words (M = 99.61, SD = 17.46) compared with the non-ecstasy users (M = 116.05, SD = 17.62), a large magnitude effect, g = -.92. The Learning over Trials (LOT) measure also provided a measure of total recall however it takes into account differences on immediate memory (Trial 1). As seen in Figure 7.5, The ecstasy users performed significantly poorer (M = 50.12, SD = 16.14) on LOT than the non-ecstasy users (M = 59.05, SD = 16.32) although this effect, g = -.54, was smaller in magnitude compared with the Total Recalled score once differences on Trial 1 had been considered.

Measures of retention; delayed recall, recognition and forgetting rate

As seen in Figure 7.6 the ecstasy users recalled significantly fewer words after the 25 minute delay (M = 13.29, SD = 3.21) relative to the non-ecstasy users (M = 16.64, SD = 3.25), and the effect size for this difference was large, g = -1.02. As seen in Figure 7.7, the corresponding Forgetting Rate scores indicated that the ecstasy users lost significantly more words between the final learning trial (Trial 8) and the Delayed recall relative non-users. Notably, these effects were independent of the presence of cannabis use. Indeed, for the non-significant main effect of Cannabis on delayed recall score, and power analysis revealed that 972 participants would be required to reliably identify an effect of this magnitude (g = .18) as significant. There were no drug related effects for Recognition, with all groups correctly identifying an average of 39 out of a possible 40 items (Ecstasy-present: M = 39.60, SD = .72, Ecstasy-absent: M = 39.72, SD = .73, g = .15). Additionally there were no significant differences between ecstasy users and non-users in false alarm rate on the recognition tests, F (1, 64) = .08, p = .75.

Table 7.9

Means, (Standard Deviations) and 2 (Ecstasy; Present, Absent) × 2 (Cannabis; Present, Absent) ANOVA Results for Memory Measures on the Unrelated List Learning Task

Measure	Descriptives				ANOVA					
					Cannabis		Ecstasy		Ecstasy× Cannabis	
	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)	*F*	*p*	*F*	*p*	*F*	*p*
Trial 1	7.85 (3.31)	6.40 (2.50)	6.31 (2.08)	6.06 (1.81)	1.88	.175	2.29	.135	0.92	.339
Total Recall	120.30 (13.68)	111.80 (15.60)	97.81 (21.13)	101.41 (19.15)	0.33	.567	14.87	**.001**	2.01	.161
Delay	17.55 (2.37)	15.73 (3.19)	13.00 (3.38)	13.59 (3.9)	0.60	.438	18.09	**.001**	2.33	.131
Forgetting Rate	0.45 (1.67)	2.0 (2.56)	2.87 (2.65)	2.82 (2.10)	1.88	.175	8.84	**.004**	2.15	.148
Recognition	39.85 (0.48)	39.60 (0.82)	39.50 (1.03)	39.71 (0.47)	0.22	.884	0.52	.475	1.765	.190
Learning Over Trials	57.50 (17.95)	60.60 (12.84)	47.31 (16.73)	52.94 (16.03)	1.22	.273	5.11	**.027**	0.10	.750
Subjective Clustering	16.23 (8.03)	13.66 (8.92)	8.52 (5.96)	9.05 (6.18)	1.53	.221	5.46	**.023**	3.15	.081

Note: Degrees of Freedom for *F* are 1, 64 for all analyses

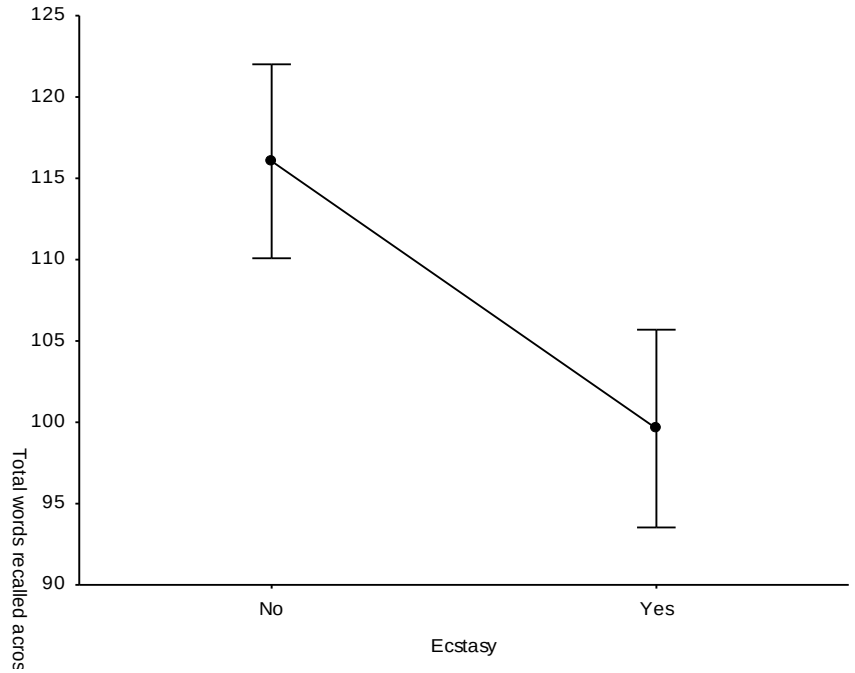

Figure 7.4. Mean total words recalled across the eight unrelated word list trials, for ecstasy and non-ecstasy users (error bars represent standard errors.)

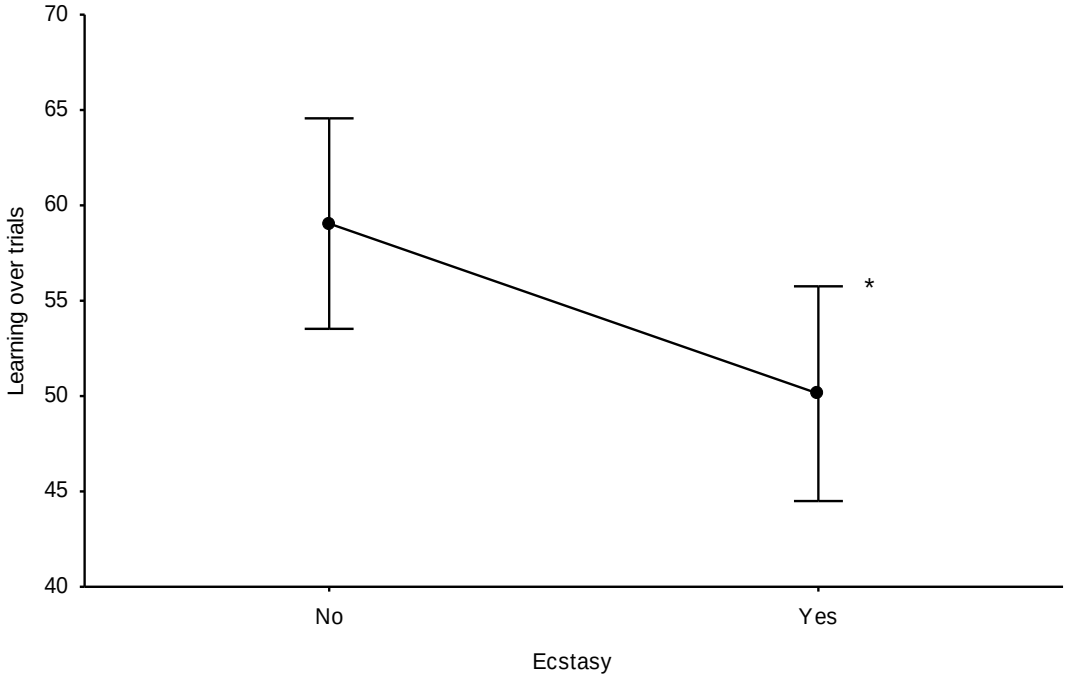

Figure 7.5. Mean learning over trials for ecstasy and non-ecstasy users (error bars represent standard errors.)

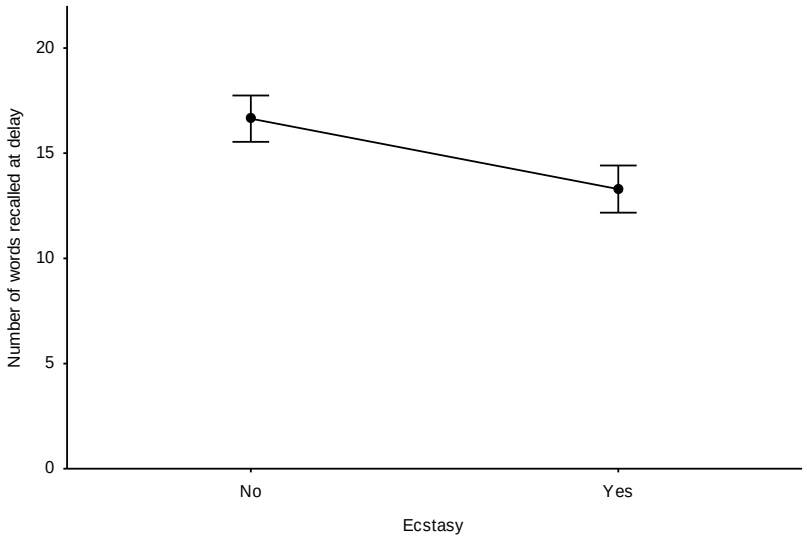

Figure 7.6. Mean words recalled in the delay trial for ecstasy and non-ecstasy users (error bars represent standard errors.)

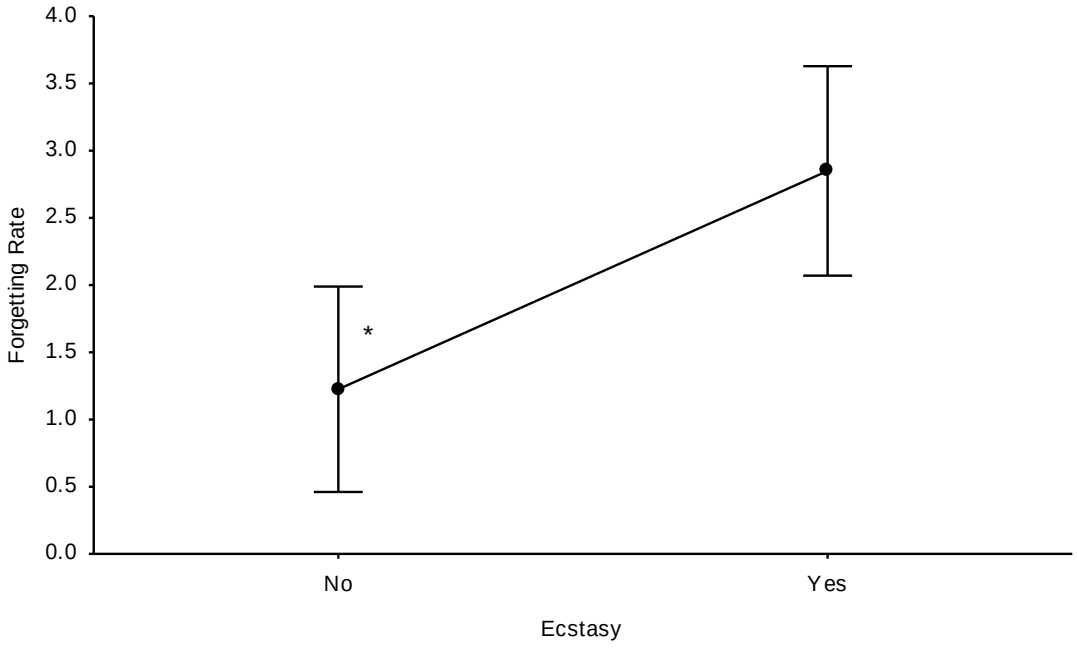

Figure 7.7. Mean forgetting rate for ecstasy and non-ecstasy users (error bars represent standard errors.)

Is ecstasy use associated with reduced ability to engage in subjective clustering?

In addition to the learning measures described above, a measure of subjective organisation was included to assess the degree to which participants organised the words in a manner that would assist their recall. A 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) ANOVA with the mean subjective clustering index for the eight learning trials as the dependent variable showed a significant main effect of moderate magnitude for ecstasy F (1, 68) = 4.89, p = .03, g = -.53, with the Ecstasy-present group clustering less (M = 9.02, SD = 7.55) than the Ecstasy-absent group (M = 13.4, SD = 7.57). There were no significant effects for Cannabis (F (1, 68) = 2.70, p = .37) or for the Cannabis x Ecstasy interaction (F (1, 68) = 2.7, p = .11). These results indicate that ecstasy users failed to utilize strategic processing of the subjective similarities of the list items to the same extent as non-ecstasy users.
In addition to examining the subjective clustering index over the learning trials, the extent to which participants used this strategy to assist with delayed recall was also assessed. 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) ANOVA with the mean subjective clustering index for Delayed recall as the dependent variable showed a significant main effect for Ecstasy F (1, 67) = 7.37, p = .009, g = -.65) a non-significant effect of Cannabis F (1, 67) = .42, p = .52, g = .15) and a significant interaction between these variables F (1, 66) = 9.01, p = .004 (Figure 7.8). Univariate breakdown analyses showed that the Drug naïve group clustered significantly more than the Ecstasy-only group, F (1, 34) = 16.85, p <.001, g = 1.31), however for those participants that used cannabis, there was no difference in clustering as a function of ecstasy use, F (1, 32) = .040, p = .84, g = .06, and for the Ecstasy-present group there was a trend for subjective clustering at Delayed recall to be influenced by a moderate effect of cannabis use, F (1, 32) = 3.48 p = .07, g = -.62. These results suggest that ecstasy and cannabis use are associated with poor engagement of subjective organisation at delayed recall.

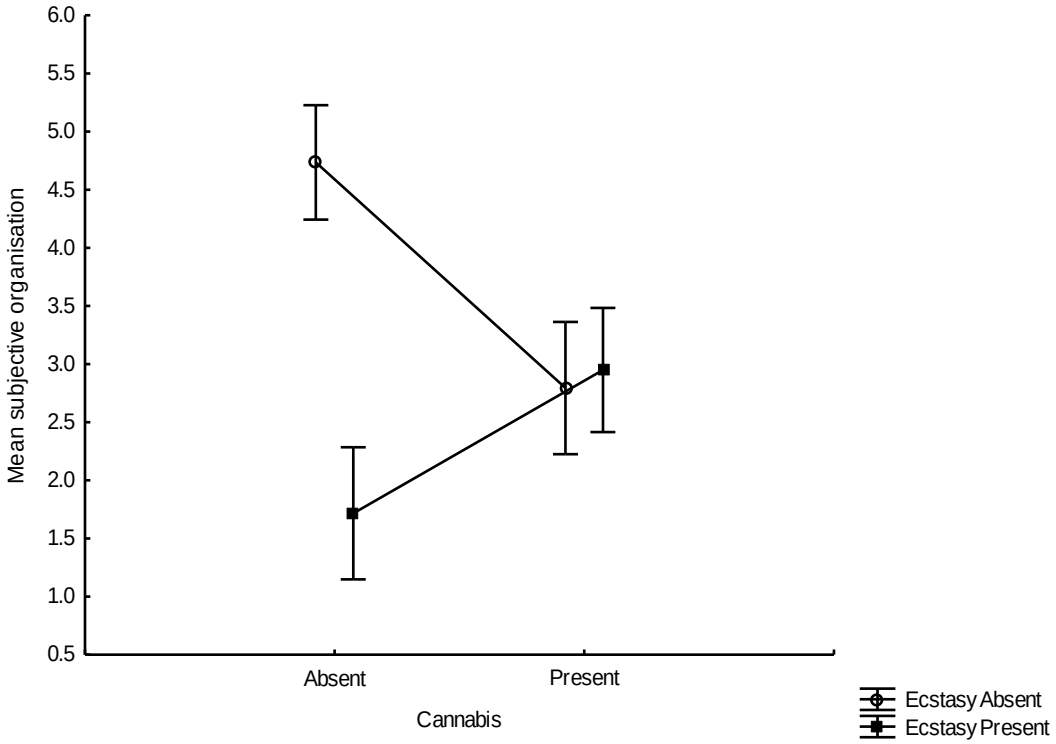

Figure 7.8. Mean rate of subjective organisation as a function of Ecstasy and cannabis use at Delayed recall (error bars represent standard errors.)

Summary

These results indicate that although both ecstasy users and non-users are improving their recall scores as a consequence of multiple list presentations, ecstasy users are taking longer to obtain a consistently high level of recall compared with non-users. Thus, although ecstasy users' scores do improve as the number of trials increase, they remain significantly lower than the others groups and would require a greater number of trials to achieve a level of recall that is comparable to the non-ecstasy users. Moreover, the overall impairment in verbal learning among ecstasy users is of considerable magnitude. Similarly the measure of forgetting rate indicated ecstasy users are performing significantly poorer than non-users, suggesting that in addition to learning fewer words over the eight trials, ecstasy users are also forgetting more words between the final learning trial and delayed recall. Finally, the inclusion of the subjective organisation measure provides valuable information

which suggests that the lower recall scores for ecstasy users may be a consequence of their lack of self- initiated organisation of the presented words.

Related list, traditional measures: The effects of ecstasy use on memory for related words.

To examine the learning curve for each of the groups, the number of words recalled on the unrelated list task was first analysed using a 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) × 8 [Trial; 1-5] mixed ANOVA. There was as expected, a significant main effect of Trial, $F(4, 64) = 204.72$, $p < .001$, with Bonferroni-adjusted pairwise comparisons showed that as the number of learning trials increased, so did the number of words recalled.

There was no effect of Cannabis on the related learning trials, $F(1, 64) = .33$, $p = .56$, $g = .14$, and power analysis revealed that 1604 participants would have been required to reliably (*Power* = .8) identify an effect of this magnitude as significant. The Cannabis × Trial, $F(4, 64) = .52$, $p = .75$, and the Trial × Cannabis × Ecstasy interactions, $F(4, 64) = .20$, $p = .96$, were also statistically non-significant.

The main effect of Ecstasy was significant and of large magnitude, $F(1, 64) = 20.23$, $p < .001$, $g = 1.1$) with ecstasy users recalling fewer words on average ($M = 13.11$, $SD = 2.43$) than non-ecstasy users ($M = 15.78$, $SD = 2.46$). This large effect for the Ecstasy-present group was independent of the effects of other drugs, with the Cannabis × Ecstasy interaction failing to achieve significance, $F(1, 64) = 2.01$, $p = .16$.

The Trial × Ecstasy interaction was not significant, $F(4, 64) = 1.25$, $p = .29$, indicating that the learning curve profiles for ecstasy users and non-users have similar patterns. However, inspection of Figure 7.9, which shows recall across the five learning trials, indicates that although the ecstasy users are improving recall on the later learning trials, they are not achieving a recall response that is comparable to the non-ecstasy users.

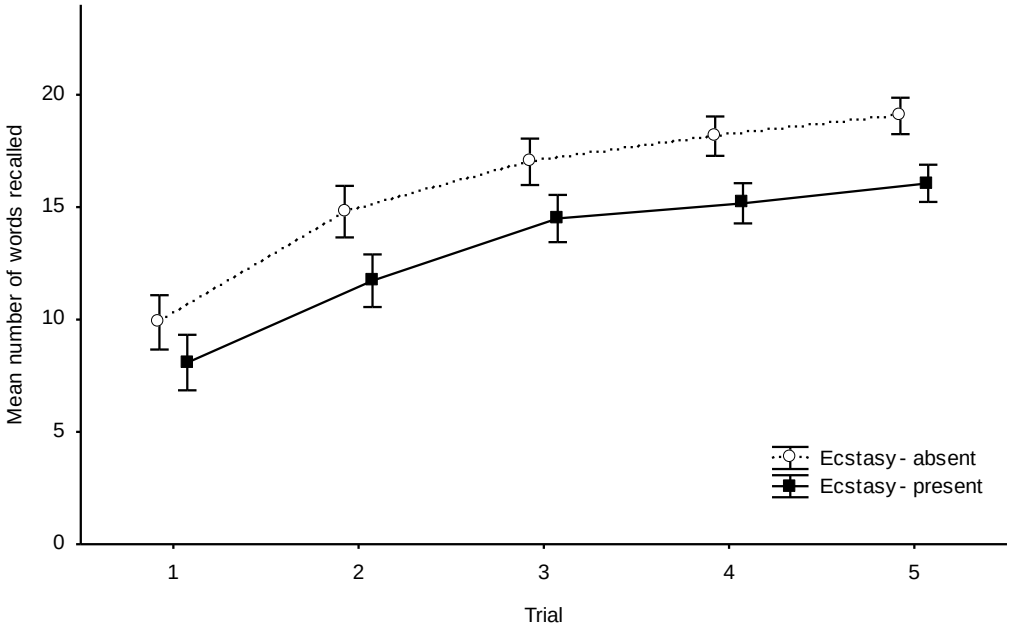

Figure 7.9. Mean number of words recalled for each trial of the related list for ecstasy users and non-users (error bars represent standard errors.)

Immediate Memory and Learning Variables

As per the unrelated list, scores on Trial 1, Total Recall and Learning over Trials (LOT) were calculated for the related list, and subjected to a 2 (Ecstasy; present, absent) x 2 (Cannabis; present, absent) ANOVA. These results are presented in Table 7.10.

Table 7.10

Means, (Standard Deviations) and 2 (Ecstasy; Present, Absent) × 2 (Cannabis; Present, Absent) ANOVA Results for Memory Measures on the Related List Learning Task

Measure	Descriptives				ANOVA					
	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)	Cannabis		Ecstasy		Ecstasy× Cannabis	
					F	*p*	*F*	*p*	*F*	*p*
Trial 1	9.95 (4.12)	9.80 (3.91)	7.88 (3.26)	8.29 (2.59)	0.02	.877	4.28	**.042**	0.10	.743
Total Recall	80.75 (8.42)	77.07 (11.61)	65.19 (14.68)	65.88 (13.71)	0.25	.616	20.29	**<.001**	0.54	.464
Learning Over Trials	31.00 (13.50)	28.06 (11.03)	25.81 (11.46)	24.41 (11.15)	0.55	.460	2.30	.134	0.06	.793
Semantic Clustering	7.84 (2.35)	7.08 (2.79)	5.15 (2.36)	4.71 (2.76)	0.90	.23	16.37	**<.001**	0.06	.80

Note: Degrees of Freedom for *F* are 1, 64 for all analyses

The main effect of Cannabis was not significant for any of the related list measures. For Total recall, the dependent measure with the largest effect size, the cannabis effect was small in magnitude, g = -.18, and power analysis showed that 980 participants would be required to reliably detect a difference of this small magnitude as significant. As shown in Table 7.10, Cannabis was also not involved in any significant interactions.

In contrast to the unrelated list performance, on the related list ecstasy users recalled significantly fewer words at Trial 1 (M = 8.08, SD = 3.55) than non-users (M = 9.87, SD = 3.58), although this effect size (g = -.49) was relatively modest compared with some of the other measures. The magnitude of this effect was similar to that identified for unrelated words (g = -.36). To examine whether the effect of ecstasy on Trial 1 performance was generalised across lists or reflecting a particular impairment when categorical properties are present in a word list, a an Ecstasy × Cannabis × List Type ANOVA was performed with Trial 1 as the dependent variable. The main effect of Ecstasy was significant, $F(1,64) = 3.98$, p =.050, with ecstasy users performing more poorly than non-users overall, though this effect was regardless of List type, with the Ecstasy x List type interaction, shown in Figure 7.10, not significant, $F(1,64) = 1.85$, p =.18. There was no significant Cannabis (p=.60) main effect nor, Cannabis × List Type (p=.12), Cannabis × Ecstasy (p = .52), or Cannabis × Ecstasy × List Type interactions (p =.62).

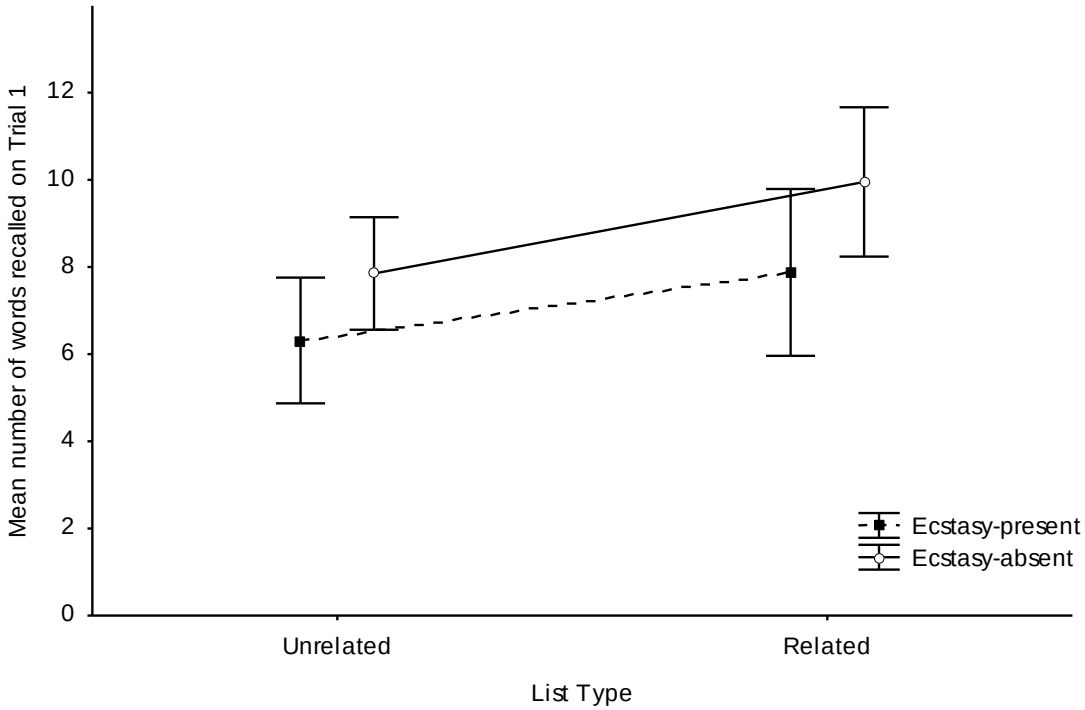

Figure 7.10. Mean number of words recalled for Trial 1 for ecstasy users and non-users on the related and unrelated word lists (error bars represent standard errors.)

Returning to performance on the related word list, there was a very large and significant effect of Ecstasy on the total number of words recalled, with ecstasy users (*M* = 65.53, *SD* = 12.12) recalling fewer words than non-users *(M* = 78.90, *SD* = 12.24, *g* = -1.10. However, there was no statistically significant effect of Ecstasy on the Learning over Trials score, *F* (1, 68) = 2.30, *p* =.134, *g* = -.36, despite ecstasy users scoring significantly lower on this index for the nonrelated list (*g* = -.54). LOT provides an index of the extent to which participants benefit from repeated list presentations, relative to Trial 1 performance. As such, the absence of the usual ecstasy related effect may be attributable to ecstasy users benefitting to the same extent as the non-ecstasy users on the related list. However, as the LOT measure is calculated by subtracting five times the Trial 1 score from the total recall, and the ecstasy present group's Trial 1 score was significantly lower than the ecstasy-absent group's score, the lack of ecstasy related effect may be reflective of their lower Trial 1 score.

Is ecstasy use associated with reduced ability to engage in semantic clustering?

ANOVA with the mean list based clustering index for the five learning trials as the dependent variable showed a significant main effect of large magnitude for Ecstasy F (1,64) = 16.37, p<.001, g = -.97, with the Ecstasy-present group clustering less (M = 4.93, SD = 2.56) than the Ecstasy-absent group (M = 7.46, SD = 2.59) overall. There were no significant effects for Cannabis (F (1, 64) = .90, p = .34, g = .23) or for the Cannabis x Ecstasy interaction (F (1, 64) = .06, p = .80). These results indicate that ecstasy users failed to utilize strategic processing of the semantic properties of the related list items to the same extent as non-ecstasy users, suggesting impaired functioning of frontal systems.

Summary

These results indicate that as with the unrelated list, although both ecstasy users and non-users are improving their recall scores as a consequence of multiple list presentations, ecstasy users are unable to obtain a level of recall that is comparable to non-ecstasy users over the five learning trials. There are variations in the way ecstasy users responded to the different list characteristics, with the magnitude of the effect for Trial 1 being larger for the related (g = -.49) than the unrelated list (g = -.36) list. There was also considerable difference in the magnitude of the effects for clustering, with the subjective organisation of unrelated list stimuli showing a moderate effect of ecstasy (g = -.57) although the semantic clustering index showed a large magnitude deficit for ecstasy users (g = -.97). The Learning over Trials measure also showed a non-significant effect for ecstasy users on the related list (g = -.36) however for the unrelated list, although the effect was lower in magnitude relative to some of the other summary measures (g = -.54) this effect was significant.

Gained and Lost Access: Unrelated List

To allow a more detailed consideration of inter-trial performance, ‘gained’ versus ‘lost’ access to items was examined. Gained access was the number of words recalled on a given trial that were not recalled on the previous trial, and lost access was the number of words not recalled on a given trial that were recalled on the

previous trial. A 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) × 7 [Interval; 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8] was performed separately for each of the gained and lost access dependent variables.

Gained access

Mean number of words gained between trial intervals for each of the drug groups are presented in Table 7.11 below. The effect of Interval was significant, $F(6, 64) = 29.73$, $p < .001$, with pairwise comparisons showing that participants gained significantly more words between intervals 1-2 and 2-3 than all other intervals, with all other intervals not significantly differing from one another.

There was a significant and moderate magnitude main effects of Ecstasy $F(1, 64) = 5.68$, $p = .02$, $g = -.57$ with ecstasy users gaining significantly more words ($M = 3.94$, $SD = .77$) between intervals than non-ecstasy users ($M = 3.33$, $SD = .78$) overall. There was no significant effect of Cannabis, $F(1, 64) = 0.86$, $p = .0.36$, $g = -.22$, and the Cannabis × Ecstasy interaction was not significant, $F(1, 64) = 1.84$, $p = .18$, as were all higher order interactions; Interval × Cannabis, $F(6, 64) = .77$, $p = .58$, Interval × Ecstasy, $F(6, 64) = 1.2$, $p = .31$, and Interval × Cannabis × Ecstasy, $F(6, 64) = .48$, $p = .82$.

Table 7.11

Means (and Standard Deviations) for Number of Words Gained Between Each Trial Interval for the Unrelated List

Interval	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)
Trial 1-2	5.80 (1.94)	6.20 (1.66)	5.19 (1.64)	6.12 (2.39)
Trial 2-3	4.10 (1.86)	5.13 (1.88)	4.38 (1.40)	5.47 (1.84)
Trial 3-4	3.35 (1.56)	3.87 (1.96)	4.19 (1.27)	4.29 (1.72)
Trial 4-5	2.50 (1.53)	3.40 (1.63)	3.31 (1.49)	3.18 (2.21)
Trial 5-6	2.55 (1.90)	3.33 (1.8)	3.69 (1.78)	3.59 (2.37)
Trial 6-7	2.20 (1.19)	2.47 (1.50)	3.81 (2.45)	3.81 (2.32)
Trial 7-8	1.75 (1.20)	2.60 (1.80)	2.44 (1.26)	2.35 (2.12)

Lost Access

Mean number of words lost between trial intervals for each of the drug groups are presented in Table 7.12 below. There was a significant main effect of Interval, F (6, 64) = 2.29, p = .041, although pairwise comparisons showed that the only difference between intervals was a trend (p = .057) for participants to lose more words between trials 2-3 compared to 1-2.

Table 7.12

Means (and Standard Deviations) for Number of Words Lost Between Each Trial Interval for the Unrelated List

Interval	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)
Trial 1-2	1.25 (1.07)	1.47 (0.99)	2.13 (1.02)	2.71 (1.76)
Trial 2-3	2.00 (1.41)	2.87 (2.03)	2.31 (1.58)	3.06 (1.92)
Trial 3-4	1.95 (1.73)	2.53 (1.55)	3.06 (1.57)	2.71 (1.65)
Trial 4-5	1.80 (1.32)	2.33 (1.34)	2.25 (1.53)	2.41 (1.62)
Trial 5-6	1.90 (1.52)	2.67 (1.35)	3.31 (1.92)	2.59 (1.97)
Trial 6-7	1.64 (1.35)	2.20 (1.32)	2.44 (1.46)	2.35 (1.54)
Trial 7-8	2.50 (1.15)	2.27 (0.88)	2.50 (1.71)	2.35 (1.76)

The effect of Cannabis was not significant and was accompanied by a small effect size, F(1, 64) = 2.01, p = .16, g = .33, and power analysis revealed that 280 participants would have been required to reliably (power = .8) identify an effect of this magnitude. The Interval × Cannabis interaction was also not significant, F(6, 64) = .79, p = .56.

There was a significant effect of Ecstasy which was moderate in size (F (1, 64) = 7.75, p = .007, g = -.67). The Ecstasy-present group lost more words between intervals (M = 2.58, SD = .71) than the Ecstasy-absent group (M = 2.01, SD = .72) overall.

This Ecstasy effect occurred regardless of the effects of Cannabis or Interval, as the Ecstasy × Cannabis, $F(1, 64) = 1.62$, $p = .21$, Ecstasy × Interval, $F(6, 64) = .90$, $p = .50$, and Interval × Ecstasy × Cannabis interactions, $F(6, 64) = .80$, $p = .57$, were all non-significant.

Delayed Recall

Gained and Lost Access analyses provide a very specific measure of how many words were gained and lost between Trial 8 and delayed recall. As expected, between groups analyses showed no significant gains between Trial 8 and Delay for losses however, there was a significant moderate to large effect of Ecstasy, $F(1, 63) = 9.68$, $p = .003$, $g = .75$, and a non-significant effect of Cannabis, $F(1, 63) = 1.76$, $p = .19$, $g = .45$, though this was modified by a significant Ecstasy × Cannabis interaction, $F(1, 63) = 4.13$, $p = .046$. This interaction is shown in Figure 7.11.

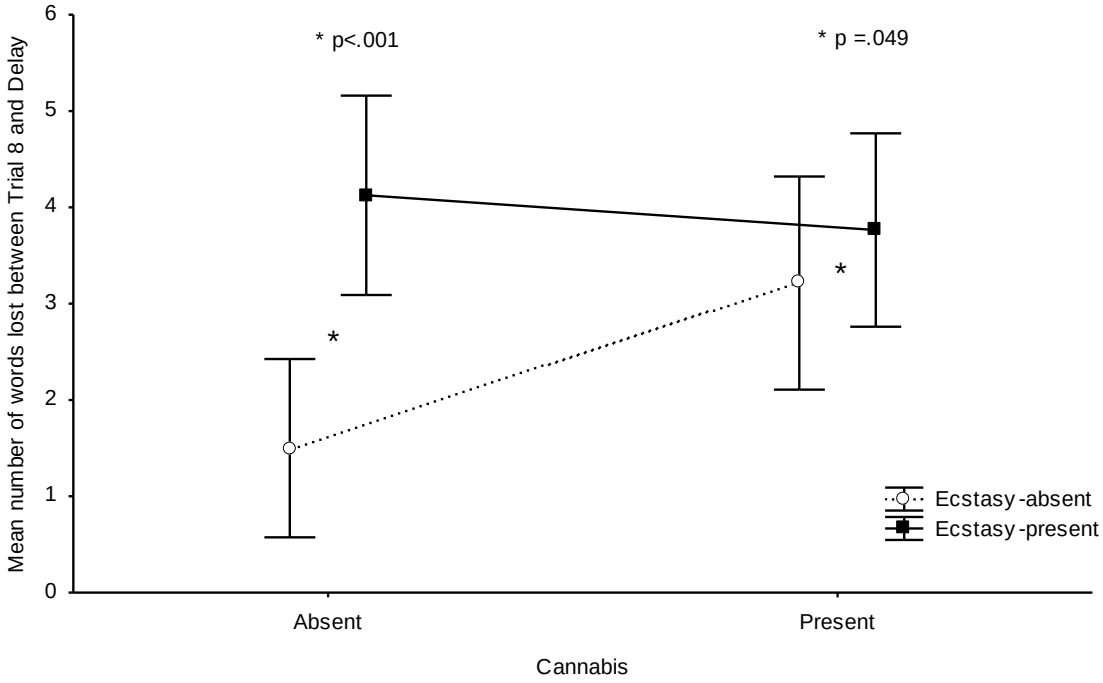

Figure 7.11. Mean number of words lost between trial 8 and delayed recall on the unrelated word list, as a function of ecstasy and cannabis use.

Figure notes: all drug groups differ significantly (p <.05) from drug naïve group (error bars represent standard errors.)

Univariate breakdown analyses showed that the Drug Naïve group lost significantly less words than the Ecstasy-only group, $F(1, 34) = 16.17$, $p < .001$, $g = 1.32$, the Cannabis-only group, $F(1,33) = 5.14$, $p = 0.03$, $g = .89$, and the Ecstasy and Cannabis group, $F(1,35) = 9.87$, $p = .003$, $g = 1.50$. Thus all drug groups lost more words in the delay compared with the Drug Naïve group. The effect sizes indicate that the Ecstasy-only group ($g = 1.35$) contributed more to the very large Ecstasy-plus Cannabis effect ($g = 1.50$) than the effect of Cannabis ($g = .89$), which was considerably smaller than the ecstasy related effects. For people who did smoke cannabis, there was no difference in the number of words lost as a function of ecstasy use, $F(1, 29) = .47$, $p = .50$, $g = .21$.

Summary

On all measures of lost access for the unrelated list, ecstasy users performed significantly poorer than non-users. This was particularly noticeable in the lost access between Trial 8 and delay, with very large effect sizes indicating more access is lost in ecstasy consuming groups than non-users, when there is delayed recall.

Gained and Lost Access: Related List

As per the unrelated list a 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) × 4 [Interval; 1-2, 2-3, 3-4, 4-5] ANOVA was performed separately for gained and lost access.

Gained Access

Mean number of words gained between intervals on the related list are presented in Table 7.13. There was no significant effect of Cannabis, $F(1, 64) = .03$, $p = .86$, and Cannabis was not involved in any interactions; Ecstasy × Cannabis, $F(1, 64) = .40$, $p = .53$, Interval × Cannabis, $F(3, 64) = 1.41$, $p = .24$, Interval × Ecstasy × Cannabis, $F(3, 64) = .89$, $p = .35$.

Table 7.13

Means (and Standard Deviations) for Number of Words Gained Between Each Trial Interval for the Related List

Interval	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)
Trial 1-2	6.35 (2.00)	5.86 (2.10)	5.75 (2.20)	5.35 (1.96)
Trial 2-3	3.65 (2.05)	3.73 (2.40)	5.06 (1.65)	4.76 (1.50)
Trial 3-4	2.05 (1.87)	2.60 (2.02)	2.93 (1.94)	3.88 (1.73)
Trial 4-5	1.10 (1.52)	1.80 (1.52)	3.31 (1.85)	2.58 (1.17)

There was a significant effect of Interval, $F(3, 64) = 58.41$, $p < .001$, and a significant effect of Ecstasy, $F(1, 64) = 9.56$, $p = .003$, $g = .74$, though as depicted in Figure 7.12, these variables were involved in a significant interaction, $F(3, 64) = 4.84$, $p = .004$). Follow up analyses showed no difference between ecstasy and non-ecstasy using groups in the number of words gained between intervals 1-2 and 3-4, however ecstasy users gained significantly more words than the non-users between intervals 2-3 and 4-5. As shown in the figure, by the 4-5 interval, ecstasy users were still gaining an average of three words, whereas the Ecstasy-absent group were only gaining one.

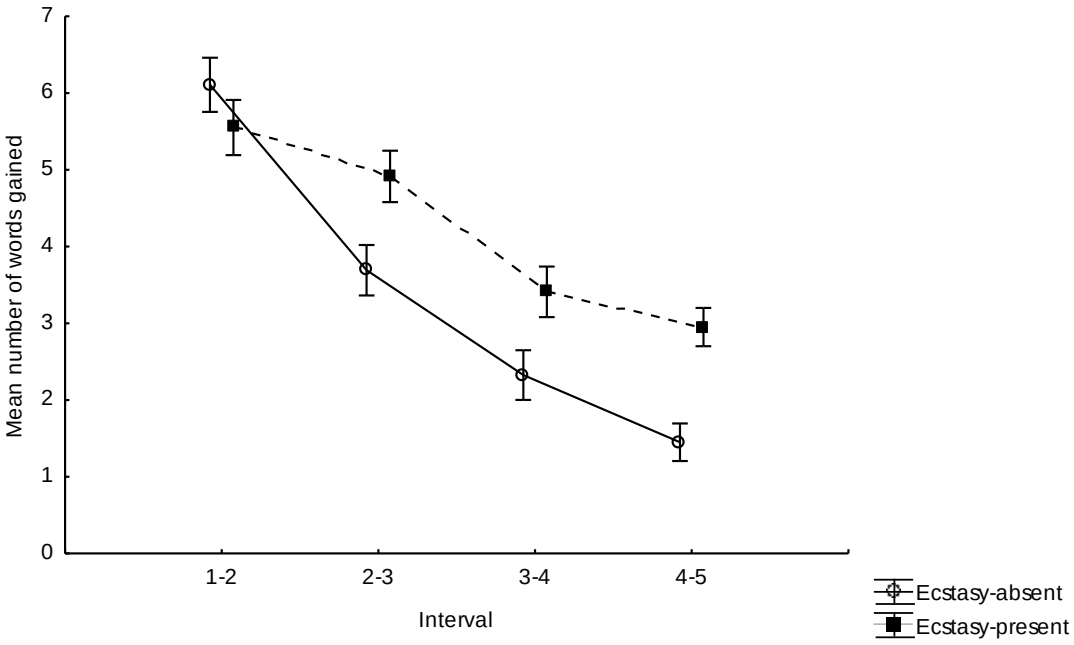

Figure 7.12. Mean number of words gained across each interval ecstasy users and non-users on the related list (error bars represent standard errors.)

Such differences in gains at the final interval may be because the ecstasy group was slower to encode and consolidate words, and therefore did not make significant gains until the later learning trials. It may also be because a larger proportion of the non-ecstasy group had reached the maximum recall (i.e. were recalling all words, by this point. This is demonstrated in Figure 7.13, which shows that more drug naïve participants had reached maximum recall.

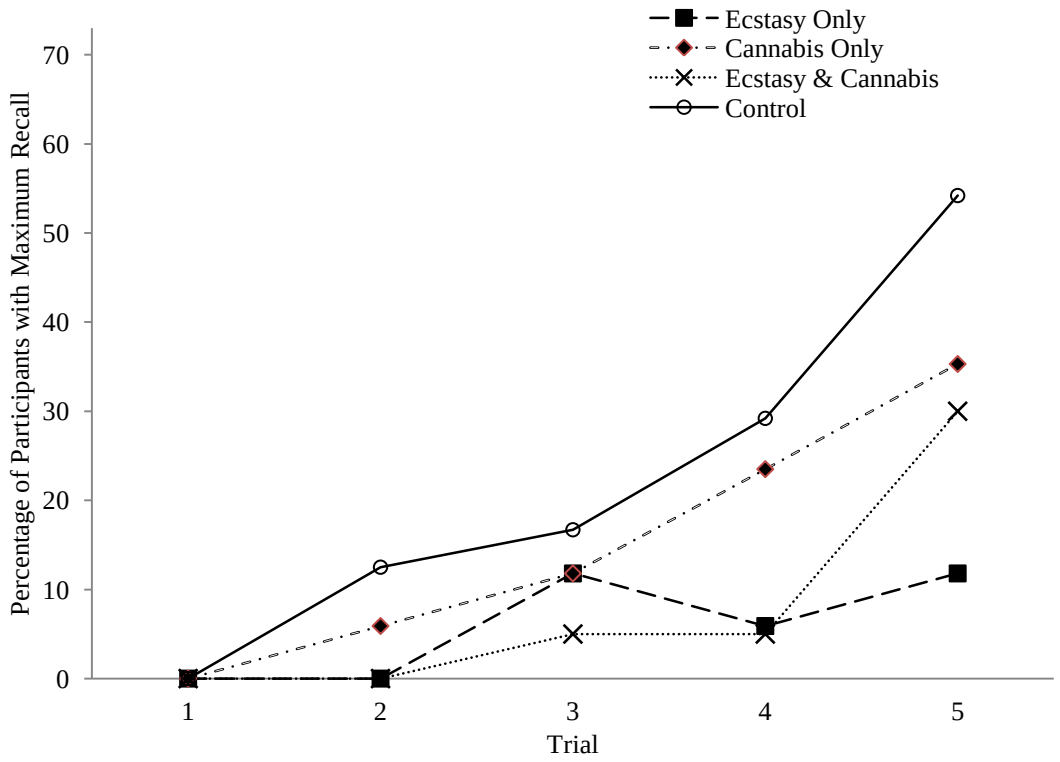

Figure 7.13 Percentage of participants reaching maximum recall across each trial for all four experimental groups

Lost Access

Mean number of words lost between intervals on the related list are presented in Table 7.14. The effect of Interval was significant, $F(3, 64) = 4.22$, $p = .007$. All participants lost more words between intervals 1- 2 and 2-3 compared with 3-4 and 4-5. The effect of Cannabis was not significant, $F(3, 64) = .21$, $p = .65$, $g = .11$, and power analysis showed that 2598 participants would be required to reliably detect a difference of this small magnitude. The Interval × Cannabis, $F(3, 64) = .15$, $p = .92$), Interval × Ecstasy, $F(3, 64) = 2.02$, $p = .11$, and Interval × Cannabis × Ecstasy interactions, $F(3, 64) = 1.25$, $p = .29$, interactions all failed to reach statistical significance.

Table 7.14

Means (and Standard Deviations) for Number of Words Lost Between Each Trial Interval for the Related List

Interval	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)
Trial 1-2	0.90 (1.41)	1.46 (1.55)	1.93 (1.38)	1.88 (0.99)
Trial 2-3	1.55 (1.80)	1.40 (0.91)	1.93 (1.94)	2.35 (1.27)
Trial 3-4	0.90 (1.12)	1.46 (1.45)	3.00 (1.93)	2.47 (1.84)
Trial 4-5	0.50 (0.60)	0.53 (0.74)	1.93 (1.53)	1.88 (1.65)

The magnitude of the main effect of Ecstasy was large and significant, $F(1, 64) = 25.14$, $p < .001$, $g = 1.20$. As depicted in Figure 7.14, ecstasy users lost, on average, significantly more words per interval ($M = 2.17$, $SD = .90$) than non-users ($M = 1.09$, $SD = .90$). The Ecstasy × Cannabis interaction was not significant ($F(3, 64) = .51$, $p = .48$) indicating that this large effect of Ecstasy occurred irrespective of Cannabis use.

Summary

The ecstasy consumers made significantly more gains between trials 2-3 and 4-5, and lost significantly words between all intervals relative to non-ecstasy users. These results may reflect ceiling effects for the control participants, such that they had less unrecalled words, and therefore had fewer words to gain compared to the ecstasy-present group. Alternatively, when the losses are taken into account for the ecstasy group, these results suggest that although ecstasy users are able to make significant gains of words between pairs of trials, they tend to do so at the expense of other words.

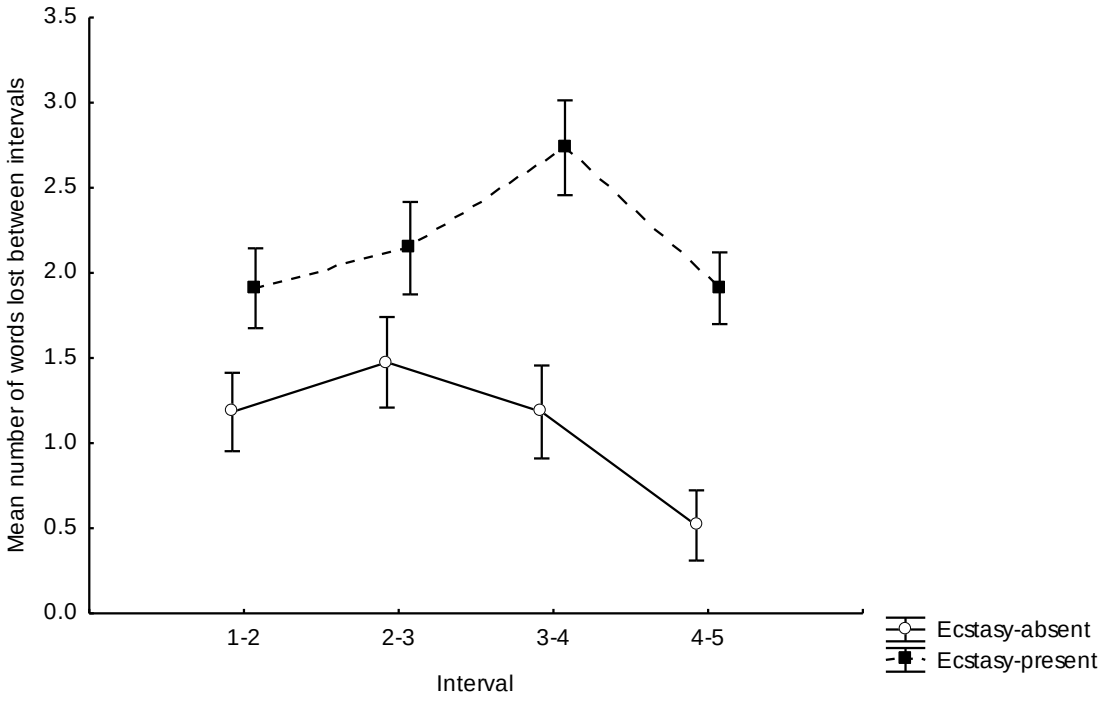

Figure 7.14. Mean number of words lost across each interval ecstasy users and non-users on the related list (error bars represent standard errors.)

Levels of Forgetting: Unrelated List

For the unrelated list, which had 8 recall trials, there are a possible 7 levels of forgetting. To examine the mean number of words forgotten at each level, 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) ANOVAs were performed for each level of forgetting. The ANOVA results, along with the mean number of words forgotten at each level for each drug group are summarized in Table 7.15 below.

For Level 1, a significant and moderate to strong magnitude (g = .74) main effect of Ecstasy was found, with ecstasy users forgetting significantly more words (M = 6.71, SD = 3.05) than the Ecstasy-absent group (M= 4.41, SD = 3.08). There was no significant effect of Cannabis and power analysis revealed that 220 participants would have been required to reliably identify an effect of this magnitude (g = .38).

The Ecstasy x Cannabis interaction was also not significant, indicating the tendency for ecstasy users to forget a word that had previously been recalled once occurred independently of other drug use.

Table 7.15

Means, (Standard Deviations) and 2 (Ecstasy; Present, Absent) × 2 (Cannabis; Present, Absent) ANOVA Results for Each Level of Forgetting for the Unrelated List

Level of Forgetting	Descriptives				ANOVA					
	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)	Cannabis		Ecstasy		Ecstasy× Cannabis	
					F	*p*	*F*	*p*	*F*	*p*
Level 1	3.70 (2.10)	5.13 (2.41)	6.25 (3.43)	7.18 (3.98)	2.50	.118	9.49	**.003**	0.11	.735
Level 2	2.80 (1.36)	4.07 (1.94)	5.00 (2.58)	4.29 (1.53)	0.37	.543	7.02	**.010**	4.63	**.035**
Level 3	3.20 (2.16)	2.93 (1.58)	3.44 (2.36)	2.88 (1.83)	0.69	.408	0.03	.851	0.85	.771
Level 4	1.40 (1.18)	2.40 (1.35)	1.44 (0.96)	2.06 (1.63)	6.46	**.013**	0.22	.635	0.35	.555
Level 5	0.90 (0.78)	1.00 (1.30)	0.69 (0.87)	1.00 (1.27)	0.62	.432	0.16	.685	0.16	.685
Level 6	0.45 (0.60)	0.33 (0.61)	0.75 (0.85)	0.59 (0.61)	0.70	.403	2.81	.098	0.01	.892
Level 7	0.35 (0.48)	0.13 (0.35)	0.06 (0.25)	0.24 (0.56)	0.04	.838	0.75	.388	3.32	.073

Notes: Degrees of Freedom for *F* are 1,64 for all analyses

At Level 2, a similar significant and moderate to strong magnitude (g = -.63) main effect of Ecstasy was found, though at this level it as moderated by a significant Ecstasy × Cannabis interaction. This interaction is shown graphically in Figure 7.15. As can be seen, by level 2, all drug users preformed more poorly, tending to forget more words that they had successfully recalled twice relative to the drug naïve controls.

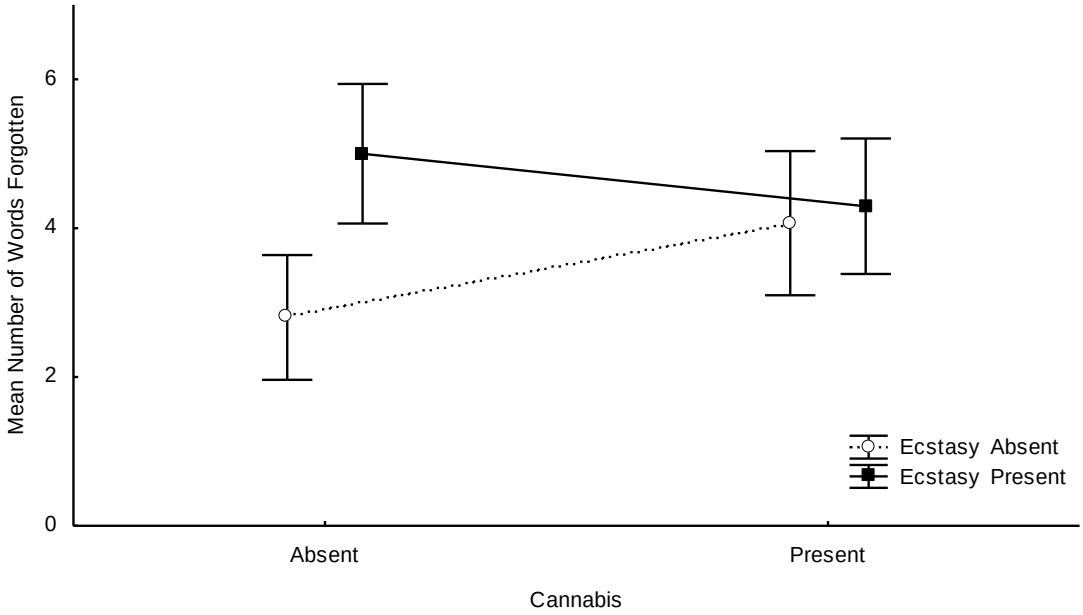

Figure 7.15. Mean number of words forgotten at level 2 for cannabis and ecstasy users and non-users (error bars represent standard errors.)

Follow-up analyses to the interaction found that among those that had not used cannabis, ecstasy users (the Ecstasy Only group) forgot significantly more words at Level 2 than those that did not use ecstasy (the Drug Naïve group), $F(1, 34) = 10.81$, $p = .002$, $g = -1.12$. However, among those that had used cannabis, there was no significant difference in the number of words forgotten between those who did and did not use ecstasy (F, (1, 30) = 0.13, $p = .714$, $g = .09$). These results suggest that ecstasy users are less likely to encode a word successfully after one previous retrieval than the other drug groups, however both ecstasy and cannabis contribute to forgetting a word that had previously recalled twice.

For Level 3, there were no significant effects for Ecstasy or Cannabis. The higher levels of forgetting need to be interpreted with caution as they are dependent on recalling the same word on more than four occasions. This degree of recall consistency was not achievable for many participants, particularly as ecstasy use was associated with a greater rate of forgetting words at level one, indicating a deficit in consolidation and hence retrieval of a previously recalled word. It is therefore possible that the ecstasy group was not able achieve the recall consistency required to obtain interpretable forgetting at higher levels. This is because, for example, Level 5 forgetting requires that the same word was consistently recalled five times. Indeed supporting this interpretation is that there were no significant effects for Ecstasy or Cannabis between levels 5 and 7. There was a significant and moderate magnitude (g = -.61) main effect for Cannabis at Level 4, suggesting that the Cannabis-present group forgot more words at level four (M = 2.22, SD = 1.30) relative to the Cannabis-absent group (M = 1.41, SD = 1.31) and this result was not modified by significant effects of Ecstasy nor an Ecstasy x Cannabis interaction.

Levels of Forgetting: Related List

For the five learning trials of the related list, there were a possible four levels of forgetting. ANOVA results and mean number of words forgotten at each level for each drug group are summarized in Table 7.16. As can be seen in Table 7.16, and as shown in Figure 7.15, the effect of ecstasy use was significant at all four levels. The magnitude of the ecstasy effect is large for level 1 (g = -.82) indicating that unlike the non-ecstasy users, for the ecstasy-present group, previously recalling a word once did not strengthen consolidation so that it might be retrieved successfully.

The effect size for level 2 was moderate (g = -.69) however the ecstasy effect was large in magnitude at level 3 (g = -.94) showing that even when a word had been recalled three times previously the ecstasy using group was more likely to forget it on a subsequent trial. This effect decreased to a moderate size at level 4 (g = -.49). The levels of forgetting analyses are largely consistent with the lost access analysis, both showing that ecstasy users have a tendency to forget more words than non-ecstasy users, both between pairs of trials, as is the case with lost access, and

throughout succeeding trials, as is the case with levels of forgetting. As demonstrated for both list types, ecstasy users were more likely to forget a word that had previously been recalled once and twice. For the related list, this effect was also apparent at levels 3 and 4, although a caveat with regard to interpretation is relevant at these higher levels.

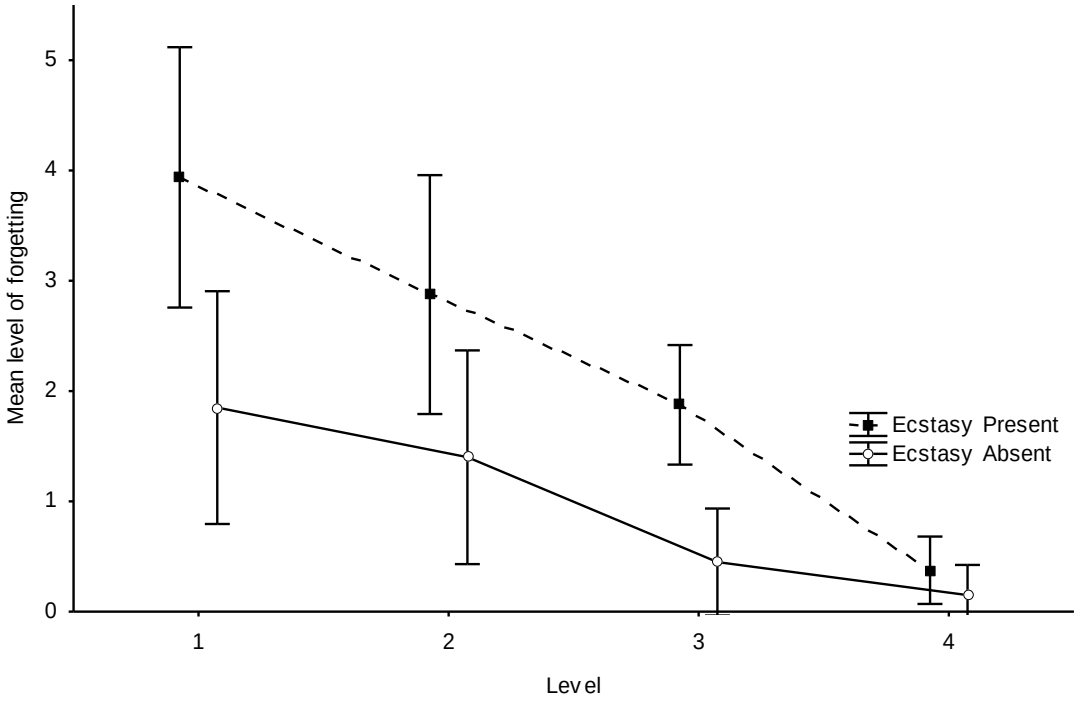

Figure 7.16. Mean number of words forgotten at each level for ecstasy users and non-users (error bars represent standard errors.)

Table 7.16

Means, (Standard Deviations) and 2 (Ecstasy; Present, Absent) × 2 (Cannabis; Present, Absent) ANOVA Results for Each Level of Forgetting for the Related List

Level of Forgetting	Descriptives				ANOVA					
	Drug Naïve Controls (n=20)	Cannabis only (n=15)	Ecstasy only (n=16)	Ecstasy and Cannabis (n=17)	Cannabis		Ecstasy		Ecstasy× Cannabis	
					F	*p*	*F*	*p*	*F*	*p*
Level 1	1.85 (2.05)	2.66 (1.95)	3.93 (2.61)	4.58 (2.89)	1.56	.216	11.65	**.001**	0.02	.888
Level 2	1.40 (1.75)	1.26 (1.48)	2.87 (2.52)	2.41 (1.46)	0.43	.512	8.40	**.005**	0.13	.716
Level 3	0.45 (0.82)	0.66 (0.81)	1.87 (1.31)	1.29 (1.26)	0.48	.489	15.40	**.001**	2.32	.132
Level 4	0.15 (0.36)	0.06 (0.25)	0.37 (0.80)	0.35 (0.49)	0.17	.678	4.10	**.047**	0.05	.809

Notes: Degrees of Freedom for *F* are 1,64 for all analyses

Are differences on processing speed or word span contributing to the observed ecstasy related memory impairments?

To evaluate the possible influence of differences in processing speed and word span on verbal learning and memory, 2 (Ecstasy; present, absent) × 2 (Cannabis; present, absent) ANOVAs were performed with highest word span achieved (maximum = 8) as the dependent variable for the word span task, and total number correct and mean reaction time (in milliseconds) as the dependent variables for the letter comparison task. Neither task scores were found to differ between groups, therefore a conservative approach to analyses was undertaken. For the word span task, there were no significant differences between the Ecstasy-present (M = 5.12, SD = .75) and Ecstasy-absent (M = 5.04, SD = .76, (F (1, 64) = .15, p = .69, g = -.09) groups. The effect of Cannabis was also not significant (F (1, 64) = 3.23, p = .07, g = -.42) with the Cannabis-present group (M = 5.20, SD = .75) scoring at a similar rate to the Cannabis-absent group (M = 4.91, SD = .75). To ascertain whether differences on word span were contributing to lower scores for ecstasy and cannabis users on total words recalled on the unrelated and related lists, ANCOVA was performed and revealed that a significant effect of ecstasy remained after controlling for word span on the unrelated (F(1, 63) = 15.57, p <.001) and related list (F(1, 63) = 22.59, p <.001) and the cannabis effect remained non-significant for the unrelated (p = .39) and related lists (p = .32).

For the letter comparison task, there were no significant differences in number correct for the Ecstasy-absent (M = 45.44, SD = 4.43) and Ecstasy-present groups (M = 44.61, SD = 4.43, F(1, 64) = .60, p = .44, g = .18) and no significant differences between the cannabis absent (M = 44.98, SD = 4.41) and present (M = 45.07, SD = 4.44, F(1, 64) = .01, p = .93, g = -.02) groups. There were also no significant differences for reaction time (milliseconds) between the Ecstasy-present (M = 2600, SD = 485.16) and Ecstasy-absent groups (M = 2723.75, SD = 491.81, F (1, 68) = 1.11, p = .29, g = .25) nor the Cannabis-present (M = 2552.95, SD = 485.16) and Cannabis-absent (M = 2770.76, SD = 489.43, F (1, 68) = 3.42, p = .07, g = .44). Subsequent ANCOVA revealed a significant effect of ecstasy remained apparent for total recall after controlling for differences on speed of processing on the unrelated (F(1, 63) = 14.26, p <.001) and related lists (F(1, 63) = 19.32, p <.001)

and no significant effect of cannabis (p = .61 for the related list, p = .56 for the unrelated list). These results indicate that the ecstasy related deficits on the above list learning measures cannot be attributed to differences in overall processing speed or memory span ability.

Examining possible dose dependence effects

In order to evaluate whether there are dose dependent effects of drug use the main memory measures, bivariate correlations were conducted between Trial 1, Total and Delayed recall and clustering measures and possible covariates, including ecstasy, cannabis and methamphetamine consumption. Methamphetamine use was included in the table, as it was the most commonly used illicit drug behind cannabis and ecstasy in this sample. These are reported in Table 7.17 below. As expected, the extent of Ecstasy use was significantly negatively correlated with Total and Delayed recall and both measures of clustering, although only recent ecstasy use was related to subjective organisation. For Cannabis use, there were no significant relationships with any memory measures apart from semantic clustering, which was associated with recent cannabis use only. ANCOVA was performed and revealed that the significant effect of Ecstasy on semantic clustering remained after controlling for cannabis use in the preceding six months F (1, 67) = 15.10, p <.001.

There were no significant relationships between the memory measures and methamphetamine use. These results are in accordance with the ANOVAs and indicate that the lower scores for ecstasy users compared with non-users can best be accounted for by ecstasy, rather than cannabis or methamphetamine use.

Table 7.17

Bivariate Correlations between Ecstasy, Cannabis and Methamphetamine use and Summary List Learning Memory Measures

	Unrelated-Trial 1	Related-Trial 1	Unrelated-Total recall	Related-Total recall	Delayed recall	Semantic clustering	Subjective clustering
Lifetime occasions of ecstasy use	-.13	-.09	-.25*	-.22	.25*	-.24**	-.14
Days used ecstasy in last 6 months	-.07	-.22	-.25*	-.38**	-33**	-.39**	-.24*
Lifetime occasions of cannabis use	-.14	-.01	-.04	-.01	-.06	-.13	-.11
Days used cannabis in last 6 months	-.21	-.13	.17	-.21	-.14	-.30*	-.21
Lifetime occasions of meth. Use	-.22	-.15	-.20	-.19	-.10	-.12	-.09
Days used meth. In last 6 months	-23	-11	-20	-19	-.06	-.48	-.07

Notes: * $p < .05$, **$p < .01$

Discussion

The effects of ecstasy use on measures of learning- comparison with previous studies

The between-lists analysis examined the effect of ecstasy and cannabis use over five learning trials of the related and unrelated list and revealed participants who had used ecstasy recalled significantly fewer words on each learning trial, with the exception of Trial 1. When Trial 1 was analysed within each list type, there was no ecstasy effect for the unrelated list although for the related list the ecstasy effect was moderate (g = -.49) in magnitude and significant at p = 0.042. Trial 1 is often not included in the ecstasy and list learning research and although an ecstasy related deficit on Trial 1 has sometimes been reported (Parrot & Lasky, 1998; Fox, Toplis, Turner & Parrott, 2001; Gouzoulis-Mayfrank et al., 2000) the effects of ecstasy on this measure is more often non-significant (Bedi & Redman, 2008; Brown, McKone & Ward, 2010; Curran & Verheyden, 2003; de Sola et al., 2008; Golding, Groome, Rycroft & Denton, 2007; Halpern et al., 2004; Halpern et al., 2010; Hanson & Luciana, 2010; McCardle, Luebbers, Carter, Croft & Stough, 2004; Thomasius et al., 2003, 2006). The meta-analysis reported in Chapter 6 found an ecstasy related effect for Trial 1 (g = -.36) although this was of much smaller magnitude than the effect sizes for the other list-learning measures.

For Total recall, the number of words recalled over the learning trials, the magnitude of the ecstasy effect on the related (g = -1.10) and unrelated (g = -.92) was large, and previous studies also reported a significant ecstasy related deficit on this measure for unrelated word lists (Bolla et al., 1998; Parrott & Lasky, 1998; Reneman et al., 2001b: Thomasius et al., 2003; Quednow et al., 2006; Thomasius et al., 2006; Reneman et al., 2006; Lamers, Bechara, Rizzo & Ramaekers, 2006; Schilt et al., 2008; Schilt et al., 2010). These findings are also consistent with the meta-analysis for this measure, which found a moderate (g = -.75) effect of ecstasy for Total recall.

The consistency between the related word list findings from the current study and that from previous research using the CVLT is less clear however: while two

studies have reported significant deficits for ecstasy users compared with poly-drug users on Total recall (Semple et al., 1999; Brown, McKone & Ward, 2010), four studies have not (Medina, Shear and Cocoran, 2005; De Sola et al, 2008; Halpern 2004, 2010). There are a number of methodological and interpretative issues that may contribute to such discrepancies.

Medina, Shear and Corcoran (2005) did not report an overall deficit for their ecstasy/poly-drug group compared to a cannabis/poly-drug group. It is possible that the discrepancy with the current results on the related lists may partially reflect the effects of poly-substance use in their participants. In the Medina et al study, the ecstasy using group only reported having used an average of 12 pills in the previous 12 months and their cannabis group had a high level of cocaine use in the preceding year (54 grams compared with 12 grams for the ecstasy users). The ecstasy using group also had a relatively high incidence of cannabis use in the preceding year (mean number of joints = 504, compared with 280 for the cannabis group). This pattern of poly-drug use differs considerably from the drug groups in the present sample, in which the ecstasy-plus cannabis group had minimal exposure to other illicit drugs and only reported 2.7 *lifetime* occasions of lifetime cocaine use on average. Medina et al., statistically controlled for poly-drug use and subsequently reported a significant relationship between ecstasy use in the preceding year and verbal memory (r = -.39, p<.001) which is broadly consistent with the present results.

De Sola et al., (2008), by contrast, found no effect of ecstasy compared to cannabis use for verbal memory assessed by CVLT performance. There were, however, a number of other ecstasy-related effects identified in their sample. De Sola et al reported dose-related effects for visual memory, with higher lifetime ecstasy consumption associated with reduced Immediate (r = -0.43, p = 0.008) and Delayed (r = 0.51, p = 0.001) recall on the Rey Complex Figure Test. This test involves a copy trial of a complex figure, followed by an Immediate recall trial after three minutes in which participants are required to redraw the figure, and a Delayed recall trial after 30 minutes. When participants were divided into "heavy" (more than 100

tablets consumed) and "moderate" (less than 100 tablets consumed) sub-groups, de Sola et al., found the heavy group performed significantly worse than the moderate users and the cannabis control group on this test. They also reported a significant dose related effect of ecstasy ($r = 0.49$, $p = 0.016$) for performance on the letter-number sequencing task which a test of verbal working memory. Thus, although de Sola et al., did not find an ecstasy related effect on CVLT performance, they still argued that due to the deficits on Immediate and Delayed recall of information, the neurocognitive profile of their ecstasy using sample was consistent with alterations in the dorsolateral prefrontal cortex, medial temporal lobes and hippocampus.

Two studies by Halpern et al., (2004; 2010) have failed to find an ecstasy related impairment for Trial 1, Total and Delayed Recall on the CVLT, however they suggested the lack of statistical significance for these differences may reflect Type II error due to small sample size. Similar to De Sola (et al, 2008), Halpern et al., (2004) did report a tendency for a subgroup of 11 "heavy users" (median lifetime use = 100) to perform more poorly than non-users on the CVLT. Both the present study and Halpern et al.'s study matched the different drug groups characteristics very closely to attempt to control for differences in poly-drug use, but despite this, there are large performance discrepancies between studies, with Halpern et al., only reporting a moderate, non-significant effect for ecstasy users on Total recall ($d = -.49$) and the present study reporting a large magnitude effect ($g = -1.10$). The Ecstasy-only group in the present study and Halpern et al.'s (2010) ecstasy using group have similar poly-drug profiles, both having median lifetime cannabis usage of 10.5 and 10 occasions of use respectively, and less than 10 occasions of other illicit drug use. The present study's Ecstasy-only group had used cannabis an average of 0.38 times in the preceding 6 months, and the mean number of days abstinent from cannabis was 460 for this group. Thus their cannabis use was very minimal. Given the trends identified in De Sola et al. (2008) and Halpern et al. (2004), a likely key contribution to the discrepancy is ecstasy dose; the present study's Ecstasy-only group had more lifetime occasions of ecstasy use (median = 87.5) compared to Halpern et al.'s study (median = 43.5). It is unlikely that poly-substance use in the current sample was responsible for a spurious effect: with the exception of inhalants (nitrous oxide), all

participants had fewer than 10 occasions of illicit drug use other than cannabis and ecstasy. In the current study, the Ecstasy-plus-cannabis group had 32 lifetime occasions of use (5 in the preceding 6 months) on average. It is unlikely that inhalant use is contributing to the verbal memory deficits in the present study however, as the extent of recent use was low and inhalant use was only prominent in the Ecstasy-plus cannabis group, and ecstasy and cannabis were not associated with any interactions for Total recall on the related list. Furthermore there is limited existing literature on the long-term effects of recreational nitrous oxide use, although there is evidence that during acute intoxication there is short term memory loss, this effect subsides with discontinuation of use (Brouette & Anton, 2001). Overall, the similarly low levels of poly-substance use in the Halpern (2004; 2010) studies and the present sample, coupled with the present sample's level of ecstasy use, which although relatively low is twice as high as Halpern's sample, indicate that the discrepancy in findings between these studies may be due to ecstasy use.

Another possible contribution to the disparity between the present study's related list findings and those of de Sola et al., (2008) and Halpern et al. (2010) is the differences in task demands. In addition to the longer list length for the current task (20 items compared with only 16 in the CVLT) the current word list was presented visually, via a computer screen, rather than being read out by an experimenter as per CVLT administration. This allowed control over the duration of item presentation and the time participants had between words to memorise and/or strategise for better recall. There is also a reported superiority effect for auditorily presented words, such that words that are heard or articulated are more likely to be recalled compared to words that are seen (Craik, 1969; Levy, 1971). The order of word presentation was also different for each learning trial in the current study, which prevented participants from relying on serial order to assist with word recall. Thus, the shorter list length, greater variability in time spent memorising each word, serial organisation and the auditory presentation of the CVLT, in combination with participants who had lower lifetime occasions of ecstasy use in the Halpern et al., 2010 study compared with the present ecstasy using group, may account for the considerable differences in findings.

The Ecstasy-present group recalled fewer words on each learning trial, and subsequently had lower recall scores overall, however the ecstasy effect for Learning Over Trials (LOT) was smaller in magnitude relative to the Total recall effect sizes. LOT is an estimate of an individual's improvement over trials (Mitrushina, 2005). On the unrelated list, although scoring significantly lower on LOT, the effect size was moderate (g = -.54) whereas ecstasy use had a large effect on Total recall (g = 0.92). For the related list, there was no significant difference between ecstasy users and non-users on LOT, and the ecstasy effect was small for this measure (g = -.36). These results indicate that the Ecstasy-present group were improving over trials to a level that was comparable to control participants for the related list, however the number of words they were able to retain from Trial 1 onwards was lower, hence the lower recall scores.

The effects of ecstasy use on measures of retention- comparison with previous studies

For the unrelated list, the current study failed to find any drug related effects for Recognition and this is consistent with previous literature (Back-Madruga et al., 2003; Bedi & Reman, 2008; de Sola et al., 2008; Gouzoulis et al., 2000; Hanson & Luciana, 2010; Indlekofer et al., 2008; Wagner, Becker, Koester, Gouzoulis-Mayfrank & Daumann, 2012). Recognition is a measure of how well items have been stored in memory, and is considered to be the domain of the hippocampal component in the Working with Memory model, as it provides a strong cue that does not require strategic involvement of the frontal component (Moscovitch, 1992). This is consistent with neuroimaging data demonstrating substantial MTL involvement in recognition processes (Cabeza, Dolcos, Graham & Nyberg, 2002; Cohn, Moscovitch, Lahat & McAndrews, 2009). According to this model, in tests of free recall retrieval is guided by the frontal component to assist retrieval from MTL or other cortical areas when the cue needs to be self-initiated. Consistent with previous findings, the ecstasy related effect for Delayed recall (g = -1.02) was significant and very large in magnitude (Brown, McKone & Ward, 2010; Medina, Shear & Corcoran, 2005; Reneman, Booij, Scmand, van den Brink & Gunning, 1999; Reneman, Majoi, Schmand, van den Brink & den Heeten, 2001; Reneman et al.,

2001b; Fox, Toplis, Turner & Parrott, 2001; Curran & Verheyden, 2003; Thomasius et al., 2003; McCardle, Luebbers, Carter, Croft & Stough, 2004; Yip & Lee, 2005; Reneman et al., 2006; Thomasius et al., 2006; Schilt et al., 2010). This was also consistent with the Rogers et al., (2009) meta-analysis (d = -1.04) and the previously presented meta-analysis, which found a large magnitude effect (g = - .98) for this measure when ecstasy users were compared with poly-drug and drug naïve controls, and a moderate effect (g = -.60) when only compared with other drug users. Only one other study has reported a measure of Forgetting Rate (Quednow et al., 2006) which is the difference between the number of words recalled on the final learning trial and the number of words recalled at delay. Quednow and colleagues found the ecstasy using group lost significantly more words (M = 2.05 words) between the last learning trial and Delayed recall than the cannabis and drug naïve control groups, and the current study's findings concurred, with the ecstasy present group losing an average of 2.08 words after the delay.

In contrast with Forgetting rate, the measure of lost access between Trial 8 and Delayed recall tracks the specific words that were recalled at Trial 8, but not at the delay. It is therefore a better indicator of retention over time, as it captures losses of specific words, rather than an aggregate number. Analysis of lost access revealed that participants who used any drug lost more words between Trial 8 and delay compared to the drug naïve participants, however ecstasy users did not exhibit greater lost access than the cannabis users, suggesting that this effect is due to the combined effects of cannabis and ecstasy.

This is an interesting result, as the meta-analysis in Chapter 6 demonstrated a very large magnitude effect of ecstasy for Delayed recall when compared with poly-drug and drug naïve controls, however when the drug naïve comparison was removed, this was reduced to a moderate sized effect. It appears as though the results from the unrelated list task are reflecting this poly-drug effect for impaired delayed recall performance.

The effect of poly-substance use on verbal learning and memory in the present study

The present study specifically investigated whether it is ecstasy/poly-drug use that is responsible for the verbal memory deficits reported for ecstasy use, by comparing the Ecstasy-only and Ecstasy-plus-cannabis (poly-substance group) with Cannabis-only and a Drug naïve group. The results showed a clear pattern: there were no interactive effects between cannabis and ecstasy, as the effect of ecstasy use on the verbal learning and memory measures was consistent between those participants who did and did not also use cannabis. That is, if a participant had used ecstasy, regardless of the extent of cannabis use, they were more likely to perform poorly on the tests of verbal memory. This finding suggests that in the current sample, poly-drug use cannot account for the verbal memory deficits reported for the Ecstasy-present group. Additionally, this lack of Cannabis x Ecstasy interaction indicates there were no protective effects of concomitant ecstasy and cannabis use.

In summary, the results from the current study were in keeping with several previous findings from the ecstasy and memory literature, and were also consistent with the findings from the Rogers et al., (2009) meta-analysis which reported large magnitude effects for an ecstasy related deficit on Total and Delayed recall of the AVLT, and the current thesis' meta-analysis which demonstrated a small magnitude effect of ecstasy on Trial 1, and moderate effects for Total and Delayed recall. The lack of interactive effects for ecstasy and cannabis use is a clear indication that the memory deficits for the Ecstasy-present group in the current study are not a result of poly-substance use. To attempt to better explain these results, the discussion now turns to examining the more specific patterns of learning that are demonstrated on list learning tasks.

The contribution of acquisition, consolidation and forgetting to total recall scores for ecstasy users-evaluation of gained and lost access.

The lower Total recall scores for ecstasy users compared to non-users suggests that their level and rate of acquisition was impaired, however a finer grained analysis of the learning curve can better inform as to whether this was the case. An inter-trial assessment of list learning performance provides information that is not available in the usual summary measures. If a participant scores 4 on the first

trial and 4 on the second, this would typically be interpreted as a failure to acquire more information. However, there are different possible underlying processes that contribute to this score. It is possible that the participant recalls an entirely different set of four items on the second trial, which suggests that they have been able to gain four items. This individual is clearly able to acquire information, but this is accompanied by a failure to consolidate what was learnt on the first trial. In comparison, another participant may consistently recall the same four items – possibly indicating that consolidation is intact but there is no acquisition from one trial to the next. By following which items are recalled from one trial to the next, it is possible to evaluate the separate contributions of failure of acquisition (assessed by gains) and failure of consolidation (assessed by losses).

For the unrelated list, there were more gains made between trials 1-2 and 2-3 for all participants, and this may reflect the rapid encoding of newly encountered words in these early trials. Interestingly, for ecstasy users, more gains were made throughout the five learning trials of the unrelated list compared to the non-ecstasy users, suggesting that ecstasy use is not associated with impaired encoding/acquisition. When considering lost access however, there was a significant effect of ecstasy of moderate magnitude, with ecstasy users also losing more words between trials. These findings indicate that ecstasy users were able to encode and acquire more words as the number of trials increased, however they were unable to consolidate these words, instead they were forgotten (lost access) and replaced by different words (gained access). Thus it appears as though ecstasy use is associated with poor recall consistency; words were only gained at the expense of others. The learning curve for the ecstasy users on the unrelated list was therefore lower than the non-ecstasy users because they were not building on a consistent recall pattern from trial to trial; instead they demonstrated a “slippery” and inconsistent learning profile, suggesting ecstasy use is associated with a deficit in word consolidation, rather than acquisition. This finding also highlights some of the problems associated with summary measure of list learning tasks; the learning curve for ecstasy users looks normal, the Learning Over Trials index suggests that their recall is improving over trials, however a more detailed assessment of the learning profile indicates that

although they are gaining words with increased trials, they are also losing more words than the other groups.

For the related list, the pattern of gained and lost access for ecstasy users is more straightforward. Ecstasy users gained *more* words between Trials 2 and 3, and also between the last two trials (4-5). The increase in gains for Trials 2-3 is consistent with the lower Trial 1 scores for the Ecstasy-present group on the related list, these findings indicate the ecstasy users gained less than the non-ecstasy users initially, but then "catch up" after the first two learning trials. For the ecstasy using participants, there was a significant gain in the number of words recalled between Trials 4 and 5, which may be indicative of this group having more words remaining to be recalled compared with the non-ecstasy users. At trial 5, sixty per cent of the drug naïve, and thirty per cent of the cannabis users were achieving perfect recall. Thus, the reason for a lack of gained access for the non-ecstasy users relative to the ecstasy users is that many of the non-users were limited in the gains they could make because there were virtually no new words for them to recall between trials 4 and 5. For the related list overall, ecstasy users also exhibited significantly more lost access than non-users and this effect was very large in magnitude.

Turning to levels of forgetting, across multiple trials as opposed to pairs of trials, ecstasy users tended to forget more words at levels 1 and 2 of the unrelated list, indicating they were forgetting words that had previously been recalled once and twice consecutively. "Forgetting" implies that the information has been stored, but subsequently lost, and it is difficult to establish whether ecstasy users are really forgetting, or not encoding effectively so that the memory trace has been consolidated. Forgetting at higher levels, which requires that word has been consecutively recalled over multiple list presentations, would be more likely to indicate forgetting, as recalling a word consecutively over four trials would suggest that the word has been effectively learnt. Cannabis and ecstasy use was associated with forgetting at level 4, however forgetting at higher levels could not be adequately assessed for the unrelated list due to the low incidence of forgetting at these higher levels, which may be a result of the low incidence of participants consecutively

recalling the same words on five or more occasions. For the related list however, ecstasy use was associated with greater forgetting at all levels. The magnitude of ecstasy related effect was large at level 3 (g = -.94) and still moderate at level 4 (g = -.49), indicating that even when a word had been consistently recalled four times, ecstasy users had a tendency to forget, which would suggest a retrieval deficit. One experimenter (the author) observed that when recalling words from the related list, there was a tendency for some participants to forget an entire category during trial 4 or 5 (eg. forgetting all the words from the "types of material" category), which resulted in considerably lower recall. It is possible that this tendency has contributed to the larger magnitude effect of ecstasy use for forgetting on the related compared to the unrelated list.

Overall, ecstasy use was associated with a higher incidence of lost access, in the absence of concurrent lack of gained access, which suggest that memory deficits for ecstasy users are not attributable to deficits in memory acquisition. This is in contrast to memory in the aging literature for example, which usually reports deficits in both gained and list access for older adults (Dunlosky & Salthouse, 1996; Moulin, James, Freeman & Jones, 2004; Woodard, Dunlosky & Salthouse, 1999). This pattern of inter-trial gains, in combination with inter-trial forgetting, suggests that deficits associated with ecstasy use impact on short term consolidation. Thus, the memory traces for words were not strengthened efficiently between trials, and were therefore lost. An abundance of research points to a role of the hippocampus in the consolidation of to-be remembered items, and previous ecstasy and memory literature has reported that the profile of verbal learning memory deficits is most consistent with dysfunction in the medial temporal lobes (Fox, Toplis, Turner & Parrott, 2001; Fox et al., 2002; Gouzoulis-Mayfrank, Thrimm, Rezk, Hensen & Daumann, 2003; Wagner, Becker, Koester, Gouzoulis-Mayfrank & Daumann (2011). Neuroimaging data have also indicated a role for the hippocampus in memory performance of ecstasy users (Becker et al., 2012; den Hollander et al., 2011; Daumann et al., 2005; Jacobsen, Mencl, Pugh, Skudlarski & Krystal, 2004; Moeller et al., 2005).

The *Working with Memory model* (Moscovitch & Winocur, 2002) suggests that it is the interaction between the prefrontal and medial-temporal cortices that is crucial for the formation of memories. This interaction between the frontal and medial-temporal regions has been supported in the neuroimaging research, with co-activation of the dorsolateral prefontal cortex and perihinal cortex (Staresina & Davachi, 2006) prefrontal cortex and left MTL (Alkire, Haier, Fallon & Cahill, 1998; Johnson, Saykin, Flashman, McAllister & Sparling, 2001) and hippocampus and perihinal cortex (Strange, Otten, Josephs, Rugg & Dolan, 2002) all being significantly correlated with free recall performance. According to the model, deliberately attending to a word increases the likelihood that it will be remembered, by virtue of the modular hippocampal component which automatically registers any information that is consciously processed in to memory. The likelihood of an item being strongly encoded and retrieved however is greatly improved if the frontal component has imposed some level of organisation to the words. In this sense, the frontal component confers 'intelligence' on a comparatively modular 'stupid' hippocampal component. The more associations formed between words, via either semantic or subjective clustering, the higher likelihood of consistent recall. To ascertain the extent to which organisational strategies contributed to the learning profile of ecstasy users, semantic and subjective clustering of items were evaluated for the related and non-related tasks respectively.

Alterations in prefrontal engagement for regular ecstasy users- semantic and subjective organisation contributes to poor verbal memory

Ecstasy use was associated with a large magnitude deficit in semantic clustering on the related list, and although correlations identified recent cannabis use as a contributing factor, this effect remained when cannabis use was controlled for. This result is consistent with that of Brown, McKone and Ward (2010) who also reported poor semantic clustering for ecstasy users compared with drug naïve and cannabis using controls. For the unrelated list, there was a moderate effect of ecstasy use associated with poorer subjective clustering, and correlations showed this effect was related to recency of ecstasy use, but not lifetime use. This was an unexpected

finding; the semantic clustering strategy for the related list was embedded in the list, and as such it was presumed that this would have been a less cognitively demanding strategy to spontaneously engage with when compared to the unrelated list, which provided no obvious means to group words by. In multi-trial, free recall list learning tasks, the number of words recalled over learning trials usually increases, and this is related to the level of organisation participants impose on the words. As each participant brings their own prior knowledge of the words to the task, and creates a subjective association between them, no word list is truly unrelated (Tulving, 1977). The results from the current study suggest that ecstasy use impairs strategic organisation, however limited strategic processing still occurs, and the smaller ecstasy related effect for subjective organisation may therefore be due to ecstasy users being able to utilise their personal knowledge of associations between words to a greater extent than they can mobilise a semantic clustering strategy that is imposed on them by the list properties. In this regard, there are more rules to the clustering strategy of the related list, and it is perhaps this confinement that impaired the ecstasy users to a greater extent than the freer associations that can be generated for the unrelated list. There is some support for this from ecstasy users' performance on verbal fluency tasks, in which participants are required to generate semantically related items (such as naming different kinds of animals) and phonemic fluency tasks, which require participants to generate words beginning with the same letter. For successful performance, these tasks therefore require a strategy to assist in forming associations between words, but the task parameters are relatively loose. Ecstasy use does not consistently impair performance on these tasks (Murphy, Wareing, Fisk & Montgomery, 2009; Verbaten, 2010) however for fluency tasks that impose more rules to word generation, such as written fluency in which only words beginning with the letter "c" and that are four letters long can be generated, ecstasy related deficits are apparent (Murphy et al.,2009). Thus, it is possible that ecstasy users performed more poorly at semantic relative to subjective clustering due to the constrained nature of the strategic organisation required for the related list.

An alternative view is that the ecstasy related effect for subjective organisation was reduced because the control participants were attempting to apply

multiple strategies simultaneously to the unrelated list to assist with recall. These strategies may include mental rehearsal/repetition, visualisation and making a story out of the words. These competing strategies may have hindered control participants' ability to organise words as quickly as they did in the related list, which in turn may have increased their reliance on medial-temporal structures to consolidate the words.

Lower scores for people with frontal lobe lesions have been reported for semantic (Alexander, Stuss & Gillingham, 2008; Gersherg & Shimamura, 1995) and subjective (Alexander, Stuss, & Fansabedian, 2003; Gersherg & Shimamura, 1995) clustering indices compared with healthy controls on word list learning tasks, suggesting organisational strategies in list learning are supported by the frontal cortex. Prefrontal activations during encoding reflect the recruitment of specific executive processes that enhance encoding and retrieval of new episodic memories (Blumenfeld & Ranganath, 2007; Spaniol et al., 2009) and neuroimaging studies have demonstrated the involvement of the prefrontal cortex in semantic clustering for healthy adults. Fletcher, Shallice and Dolan (1998) used PET and a list learning paradigm that varied the degree of organisational difficulty in order to study the neural correlates of semantic clustering. During the condition that most closely matched the related list task in the current study, participants had to impose semantic clustering without being provided with a category cue. Fletcher and colleagues observed increased blood flow to the left dorsolateral prefrontal cortex during this condition, which was not apparent in the less demanding condition, in which words were already grouped in their categories. These findings were later replicated in a very similar study which used fMRI to examine the neural processes associated with semantic clustering, with the addition of observed involvement of the inferior dorsolateral prefrontal cortex (Savage et al., 2001).

Alterations to neural activity in the dorsolateral prefrontal cortex have also been reported for regular ecstasy users (Bosch et al., 2013; de Win et al., 2007; Moreno-Lopez et al. 2012) and markers of neuronal integrity have been shown to be reduced in the frontal cortex of ecstasy users, and this reduction was significantly correlated with previous exposure to ecstasy (Reneman et al., 2002). Neuroimaging

research has also linked memory updating to the dorsolateral prefrontal cortex (D'Espositio, Postle & Rypma, 2000; Jonides & Smith, 1997). Memory updating refers to processes that involve actively manipulating incoming information, and as such is not dissimilar to engaging in organisational strategies such as mentally re-ordering a list of words to enhance consolidation and recall. Following a series of studies designed to assess which component (inhibition, switching or updating) of executive functions is most affected by ecstasy use (Fisk, Montgomery, Murphy & Waring, 2004; Montgomery, Fisk, Newcombe & Murphy, 2005), Fisk and Montgomery (2008) reported that regular ecstasy users were more impaired relative to cannabis users and drug naïve controls on tasks that require memory updating. Using PET to assess regional cerebral glucose metabolism (rMRGlu) and verbal memory performance (RAVLT) Bosch and colleagues reported decreased rMRGlu in the dorsolateral prefrontal cortex, bilaterally for ecstasy users (n = 19, mean lifetime dose = 457.9) compared with drug naïve controls. The decreased metabolism in the dorsolateral prefrontal cortex was negatively correlated with lifetime ecstasy use, and negatively correlated with ecstasy users scores on Total and Delayed recall of the RAVLT. Overall, the findings from the list learning indices indicate that ecstasy users were not as successful as non-users in self-initiating organisational strategies to assist with verbal learning and this limited strategising has contributed to the lower recall scores for ecstasy users relative to cannabis and drug naïve controls.

Deficient organisational strategies and inter-trial consolidation contribute to verbal memory impairments associated with ecstasy use

The present study has identified several cognitive processes that account for ecstasy users' performance on list learning tasks. With the exception of impaired delayed recall, which was associated with poly-drug use, the drug related effects found for the various measures of list learning were related to ecstasy and not cannabis use. The Ecstasy-present group showed deficits of a large magnitude on Total recall, Forgetting rate, lost access to words and Semantic clustering. For Trial 1 and Recognition however, the ecstasy group's performance did not significantly

differ from the others, although the effect size for Trial 1 (g = -.36) was larger than that of Recognition (g = .15). Immediate memory span has limited capacity for elaborative processing as it is measured after the first list presentation, and reflects memory for the number of items that can be held in memory at one time, rather than ability to learn. Recognition tasks also require less effortful processing, as all the studies items are available at test, and participants can then rely on recent familiarity with the words to determine whether or not they were on the list. Alternatively, retrieving and learning words require a set of more complicated cognitive skills relative to a test of immediate span and recognition, suggesting that when these more demanding processes are required, the presence of a history of ecstasy consumption impairs task execution. Thus, these results suggest that for ecstasy users, basic memory span and recognition of familiar items remain intact, however organisational strategies, consolidation and retrieval of newly learnt verbal material is impaired. The finding that ecstasy use impairs the execution of these more complex operations is consistent with the suggestion that verbal memory deficits for ecstasy users are dependent on the cognitive complexity of the tasks (Brown, McKone & Ward, 2010).

Evaluation of inter-trial gains and losses indicated that the lower recall for ecstasy users compared to non-users was a consequence of losing more words between learning trials, which is a consolidation or retrieval deficit. The ability to consolidate and retrieve words relies on a network comprising hippocampal/MTL memory mechanisms that enable associative and binding processes which support consolidation, as well as cognitive control processes mediated by the dorsolateral prefrontal cortex that enable strategic encoding and retrieval processes. During encoding, the interaction between frontal and hippocampal regions provides elaborated representations of target items that can be consolidated and stored. At retrieval, these interactions act to monitor stored information and specify retrieval cues. Thus, imposing organisation on to-be remembered items at either encoding or retrieval strengthens the memory trace in the hippocampus and increases the likelihood that words will be consistently recalled. Although the ecstasy users in the current study did initiate some semantic and subjective clustering, indicating that

they were aware this could be beneficial for task performance, they were unable to engage this strategy effectively enough to strengthen consolidation, and subsequently lost access to previously recalled words.

Chapter 8

Study 3. The Effects of Ecstasy Use on Source Memory.

Summary of the previous study

The previous chapter investigated whether ecstasy use is associated with explicit verbal memory deficits, and if so, whether these deficits can be attributed to impaired strategic or storage processes equally. The results showed a clear pattern of deficient memory performance on a related and non-related list learning task for ecstasy users relative to drug naive controls, and that this result was not influenced by cannabis use. This was interpreted as indicating that lower recall scores for ecstasy users are due to a combination of reduced learning and storage capacity, as well as impaired ability to engage in strategic processes that can enhance verbal memory. Thus far, the results are suggestive of neurocognitive alterations in both the prefrontal and hippocampal networks associated with verbal learning.

This chapter continues to investigate the relative contribution of different memory processes towards the verbal memory performance of ecstasy users. The present study considers the effect of ecstasy use on source memory, which has been researched extensively in the memory literature generally, but has thus far been neglected in the ecstasy and memory literature. The following section begins with a discussion of the difference between item memory and memory for source, as well as its neural correlates. A further review of evidence for memory deficits on two different types of source memory tasks, one which is primarily a hippocampal mediated task; the other which requires more involvement from the frontal strategic component of the *Working with Memory* model is then conducted. This precedes the aim of the present study, which is to investigate whether ecstasy users perform differently to controls on these two source memory tasks which place different demands on the neural components of the working with memory model.

What is source memory?

In everyday events, multiple components of an event need to be bundled together to provide the context in which the event occurred. For example, it may be important to recall a story relayed by a friend (memory for content) and to also recall the person who relayed the story (the source of the story). Thus, memory systems are required to bind items and context together to form a complete episodic memory, and this additional contextual information allows a memory to be more deeply encoded. Binding is a cognitive process elicited by neural mechanisms that link, or associate different elements within or between a memory episode and its context (Naveh-Benjamin, 2000). Contextual information, such as remembering where, when or by whom an action occurred, tends to be forgotten, perhaps because the context is not as well attended to as the item itself. Studies of verbal episodic memory in ecstasy users have investigated memory for items, such as words, numbers or pictures. Episodic memory however involves remembering the content (or item) *and* the context in which it was perceived. Memory for context is referred to as source memory, associative memory (Naveh-Benjamin, 2000) or source monitoring (Johnson, Hashtroudi & Lindsay, 1993).

Is source memory distinct from item memory?

The distinction between source and item memory appears to have good face validity. There are many instances where there is a failure to recall the source of information in the presence of intact recollection for the target item. Because such tasks require associating multiple components from a scenario into a unitary representation, source memory performance in the laboratory is typically poorer than that of item memory scores (Craik, Moris, Moris & Loewen, 1990; Glisky, Ploster & Routhieaux, 1995; Glisky, Rubin & Davidson, 2001; Janowsky, Shimamura & Squire, 1989; Johnson, 1992; Johnson, Hashtroudi & Lindsay, 1993; Moscovitch & Winocur, 1992; Shimamura & Squire, 1987; Yonelinas, 1999). Response times for making a source memory judgement are longer than recognition (old/new) decisions (Johnson, Koumios & Nolde, 1996; Nolde, Johnson & D'Esposito, 1998) and this time discrepancy has been attributed to the recruitment of multiple, simultaneous memory processes (Johnson, Hashtroudi & Lindsay, 1993; Johnson, 2005) and to the more effortful processing requirements of recollection as opposed to familiarity

(Curran & Hintzman, 1995; Eichenbaum, Yonelinas & Ranganath, 2007; Yonelinas, 1999).

In recognition tasks, the exact item presented during the study phase is re-presented to the participant, who is required to recognise whether the item is old or new. This decision can be made using a general sense of whether or not the item was recently seen, without having to construct a detailed representation of the past episode, a process that is argued to rely on *familiarity (*Yonelinas, 1999). Source judgement tasks however, require the recollection of context-specific details that occurred at the time the item was originally presented, thus the item retrieval is supported by *recollection* of some contextual information. For example, the participant might be asked to remember whether the word "obey" was presented on the left or right side of the computer screen, or in red or blue coloured font. Source memory has been investigated using a variety of different source contexts, including: perceptual features of the stimulus item (such as upper or lower case text, font colour or auditory qualities such as words presented in a male or female voice, eg. Doerkson & Shimamura, 2001; Drag et al., 2009; Troyer, Winocur, Craik & Moscovitch, 1999; van Niekirk et al., 2004), modality information (item presented via visual or auditory means, eg. Kausler & Puckett, 1981), spatial features (location of an item on the screen or in a matrix, eg. Cansino et al., 2012; Spaniol, Madden & Voss, 2006), cognitive operations performed at encoding (eg. Davachi, Mitchell & Wagner, 2003; Dulas & Duarte, 2011), location (such as in an experimental session or from a source outside of the testing session (eg. Craik, et al., 1990), temporal aspects (time of day or the temporal sequence of items among a list, eg. Kausler & Wiley, 1990) and self information (imagining a word or speaking a word, performing an action or saying a word, eg. Cohen and Faulkner, 1989; Hashtroudi, Chrosniak, and Johnson, 1989; Hornstein and Mulligan, 2004).

Neural correlates of source memory

There is also evidence that source and item memory are subserved by different neural networks. A study of source memory in amnesic persons asked participants to recall fictitious facts about famous people and to judge whether they had learned the fact during the experiment or outside of the experiment. The authors

reported that those participants who had difficulty recalling the source of the fact also performed at a significantly lower level on tests of frontal lobe function (Schacter, Harbluk, & McLachlan, 1984). Many early studies of source memory were conducted with people with frontal lobe lesions. Janowsky, Shimamura and Squire (1989) examined memory for recently learned facts and memory for the source of the facts in persons with frontal lobe lesions and in age-matched older adults. They reported that the frontal lesioned group recalled as many facts as the controls, however they were less able to correctly identify when and where they had learned those facts. A number of investigations with normally aging older adults have supported a role for the frontal lobes in source memory. In these experiments, source memory has been found to be disproportionately impaired compared to memory for items, and this effect is larger for older adults with reduced frontal lobe function (e.g., Craik, Morris, Morris, Loewen, 1990; Glisky, Polster, & Routhieaux, 1995; Glisky, Rubin, & Davidson, 2001). Further, studies of people with frontal lesions reported that frontal damage often resulted in deficits on source memory tasks, while item memory remained at a similar level to control participants (Ciaramelli & Spaniol, 2008; Duarte, Ranganath, Knight, 2005; Johnson, O'Connor, & Cantor, 1997; Schacter, Harbluk, & McLachlan, 1984; Shimamura & Squire, 1987; Simons, Verfaellie, Galton, Miller, Hodges, & Graham, 2002). These lesion studies led some researchers to argue that it was the frontal rather than temporal lobes that were crucial for accurate source monitoring (eg. Butters, Kaszniak, Glisky, Eslinger, & Schacter, 1994; Glisky, Polster & Roiuthieaux, 1995; Glisky, Rubin & Davidson, 2001; Milner, Corsi & Leonard, 1991).

Consistent with lesion studies, neuroimaging results have provided evidence that the frontal lobes are very active during source memory performance for healthy young adults (e.g., Hayes, Ryan, Schnyer, & Nadel, 2004; Rugg, Fletcher, Chua & Dolan, 1999; Van Petten, Senkfor, & Newberg, 2000) with some research implicating the left prefrontal cortex in source monitoring (Duarte, Henson & Graham, 2011; Dulas & Duarte, 2011; Hayes, Buchler, Stokes, Kragel & Cabeza, 2011; Mitchell, Raye, Johnson & Greene, 2006). To directly compare imaging results for item and source memory, Dobbins, Foley, Schacter and Wagner (2002) required participants to learn words during two different task conditions; judging

whether they are unpleasant or pleasant, or abstract or concrete. At the time of retrieval, participants were presented with three words; one word that was new, one that was seen during the pleasant/unpleasant task and the other during the abstract/concrete learning condition. In the item retrieval condition, participants had to determine whether or not the new item was new, and for the source retrieval condition, participants were required to determine which of the study conditions the word was originally presented in. This allowed a direct comparison of brain activity associated with item and source retrieval. The left inferior prefrontal cortex showed increased activation during source encoding and retrieval, and not during item retrieval, and this activation occurred regardless of whether the source judgement was accurate. Dobbins et al. thus argued that the areas of the left PFC are required to attend to and control aspects of source memory.

Glisky and colleagues (1995) argued for a "double dissociation" between item and source memory, stating that memory for content was more reliant on the medial temporal lobes and memory for the source of the content required engagement of the frontal cortex. They classified older adults based on their performance of a battery of neuropsychological tests that assessed frontal lobe and medial temporal lobe function. Participants classified with 'high' and 'low' frontal function did not differ on a subsequent test of item memory (correct recognition of a target sentence) however the 'high' frontal function group outperformed the 'low' frontal function group on correctly identifying the source of the sentence (male or female voice). When the same participants were divided according to their medial temporal lobe (MTL) performance, the 'high' MTL function group outperformed the 'low' MTL function group for item memory, but the groups did not differ on source accuracy. Gilsky and colleagues concluded that although the MTL is involved in episodic memory generally and therefore also in source accuracy, the level of frontal involvement is more important in determining source accuracy.

However, especially important to source memory performance are processes that bind (or associate and organise) features that co-occur with the target item during encoding or retrieval. To demonstrate the role of the hippocampus in source memory, Starasina and Davachi (2008) devised an experiment that varied the extent

to which a target item was bound to its context. On each trial, a target item (eg. a shirt) and an associated context (eg. a colour) were presented under one of three different binding conditions; for the combined condition, the a blue shirt was presented, for the spatially discontiguous condition the target was presented within a blue frame (thus increasing the gap between the item and the context) and in the spatiotemporally discontiguous condition the colour was presented first, followed by a blank screen, followed by the target, thus temporally separating the target from the context. The encoding task and the type of association task (linking the target to a colour) were held constant, thus hippocampal modulation during encoding could be attributed more confidently to changes in the binding requirements of the trial. The results indicated that as the gap between the item and context increased in space and time, so did hippocampal activation during encoding. Consistent with this interpretation, Rugg and colleagues (2012) reviewed eight fMRI studies that investigated hippocampal activity during source memory tasks and concluded that hippocampal activity covaried with the degree to which source information was encoded or retrieved. Studies of episodic memory in general have long established the role of the hippocampus in this binding process (for reviews see: Bird & Burgess, 2008; Simons & Spiers, 2003; Squire, Clark, Stark & Clark, 2004; Wixted & Squire, 2011). fMRI studies have repeatedly demonstrated activation of the hippocampus during encoding to be associated with subsequent accurate source retrieval (Cansino, Maquet, Dolann & Rugg, 2002; Davachi, Mitchell & Wagner, 2003; Diana, Yonelinas & Ranganath, 2007; Dobbins, Rice Wagner & Schacter, 2003; Giovanello, Schnyer, & Verfaellie, 2009; Kensinger & Schacter, 2006; Mayes, Montaldi & Migo, 2007; Preston, Schrager, Dudukovnic & Gabrieli, 2004; Skinner & Fernandes, 2007; Starasina & Davachi, 2008; Ranganath et al., 2003) whereas increased activation of the perihinal cortex in the medial temporal lobe has been associated with item, but not source retrieval (Davachi, Mitchell & Wagner, 2003; Diana, Yonelinas & Ranganath, 2010; Kensinger & Schacter, 2006; Starasina & Davachi, 2008; Ranganath et al., 2003).

Overall, although their respective roles are not entirely clear, research to date indicates that *both* the hippocampus and the frontal cortex are required for successful encoding and retrieval of source memory. Evidence indicates that the prefrontal

cortex is needed for cognitive control processes such as attending to multiple aspects of a stimulus which involves increased deliberate monitoring of an episode (eg. Dobbins, Foley, Schacter & Wagner, 2002; Johnson, Hashtroudi, & Lindsay,1993). These frontal processes have been suggested to work concurrently with the hippocampus, which appears to be involved in the formation of rapid associations between item and context (eg. Diana, Yonelina & Ranganath, 2010; Starasina & Davlich, 2008).

Source memory ability declines with age

The formation of associations (the binding together item and context) is significantly compromised with increased age. This has been well demonstrated in studies of older adults, in which recognition memory and item memory have been shown to be more robust than source memory, which appears to be more vulnerable to the effects of aging. Age-related memory impairment begins in early adulthood (Raz, 2000; Raz et al., 2005) and has been extensively researched in older adults (usually defined as over 60).

In an early meta-analysis, Spencer and Raz (1995) reported that source accuracy was significantly compromised by increased age. Their analysis included 46 studies of source memory in older adults (mean age = 65) all of which included a younger aged control group (mean age = 35). They reported a moderate effect of age on memory for items (d = 0.58, CI: 0.48 - 0.67) and a large effect of age for source memory (d = 0.87, 95% CI: 0.78 - 0.96). The difference between these effect sizes was significant. Further, Spencer and Raz reported that age-related deficits in source memory were larger for source memory tasks where source information was only loosely associated with the target item. For example, it was more difficult for participants to recall the quadrant of the screen a word had been presented in than it was to recall the colour a word was presented in. They argued that more difficult source memory tasks were those in which there was distance between the item and the source of the item.

Old and Naveh-Benjamin (2008) conducted an updated meta-analysis comparing source memory performance between younger and older adults. They reviewed 90 experiments that included 3197 young (mean age = 21) and 3192 older participants (mean age = 70). The authors also categorised source memory tasks according to the type and difficulty of the binding processes that were required for successful task performance. In order to investigate whether some source memory tasks were more difficult to perform as a function of increased age, as suggested previously by Spencer and Raz (1995) they generated separate effect sizes for each task category. The three easier tasks were labelled; *Source memory* (memory for which word was spoken by which voice); *Context* (memory for which word appeared in which font) and *Modality* (memory for whether a word was seen or heard) and the more difficult tasks were labelled *Temporal* (memory for which word was viewed first); *Location* (memory for where on a screen the word appeared) and *Item pairs* (memory for which two words appeared together). Results showed a significant source memory deficit for older compared to younger adults, and source memory performance was significantly lower than item memory scores for the older age group. Also consistent with Spencer and Raz's earlier analysis, the age effects (weighted average effect sizes) were particularly large for the tasks that required more extensive binding processes, such as memory for Temporal order (d = 1.07, CI: 0.65-1.19), Location (d = 1.01, CI: 0.89 – 1.13) and Item pairs (d = 1.02, CI: 0.83 – 1.20). There were smaller, although still moderate effects of age for Source (d = 0.73, CI: 0.64 – 0.82), Context (d = 0.82, CI: 0.66 – 0.97), and Modality (d = 0.71, CI: 0.49 – 0.94) although older adults scores on Modality tasks did not differ significantly from their item memory performance. The meta-analysis therefore demonstrated that age related deficits on source memory tasks are distributed unequally across task categories, such that variation in the type of item to context binding appears to modulate the degree of difficulty and therefore influenced age related impairment. Consistent with the meta-analytic findings, within-subjects samples have also showed differential effects of age on different types of source information. For example, older adults performed more poorly on a temporal source task compared to a spatial location source task (Parkin et al., 1995), older adults did not differ in the number of correct source judgments for the colour of items but made significantly more errors when trying to identify the screen location in which an item

was presented (Chalfont & Johnson, 1996) and older adults made fewer correct source judgements for the spatial location of pictures, but made a similar number of correct source judgements to younger adults for whether a statement was initially presented as true or false (Siedlecki, Salthouse, & Berish, 2005).

Memory impairments in older adults have been demonstrated to be associated with neurological changes, including volumetric decreases in the hippocampus (Raz, Rodrigue, Head, Kennedy, & Acker, 2004) and frontal cortex (Giorgio et al., 2010). Studies of normal aging have also demonstrated reduced serotonin transporter in the frontal cortex, hippocampus and diencephalon (Blin et al., 1993; Goldberg et al., 2004; McEntee & Crook, 1991; Meltzer et al., 1998; Meneses, 1999; Michelsen, Prickaerts & Steinbusch, 2008; Porter, Lunn & O'Brien, 2003; van Dyck et al., 2000; Verhoeff et al., 2000; Wong et al., 1984). More recently, decreases of around 10% per year of have been reported for 5-HT 1A (Bhagwagar et al., 2004; Moller, Jakobsen & Gjedde, 2007) and 5-HT 1B (Matuskey et al., 2012) receptor binding in both the frontal and temporal cortices in healthy human subjects.

There is presently little doubt that serotonin modulates acetylcholine release of the central cholinergic system and plays a role in neurotransmission via cholinergic pathways to the hippocampus and frontal cortex and that experimental disruption to the serotonergic system, in rodents, produces spatial memory impairments (Decker & McGaugh, 1991; Richter-Levin & Segal, 1993; Steckler & Sahgal, 1995; Ruotsalainen et al., 1998). Serotonin induced neural plasticity, as shown in studies of long-term potentiation and long term depression, is believed to play a key role in the encoding and retrieving of memories (Buhot, Martin & Segu, 2000; Michelsen, Prickaerts & Steinbusch, 2008). It is plausible therefore that perturbation in serotonergic transmission may alter frontal- and hippocampal-dependent source memory, and evidence from the effects of an acute tryptophan depletion (ATD) study suggests this may be the case. McAllister-Williams, Massey and Rugg (2002) investigated the effect of ATD on source memory. They required participants to learn words that were presented in a male or female voice. At test, participants completed a recognition task (old/new judgements) and if the word was judged as old, participants were required to recall the gender of the voice that had

presented the word (source test). There were no differences between the ATD condition and placebo for recognition scores, however ATD significantly reduced the number of correct source judgements. This result mimics the age-related effect of poorer source memory relative to recognition memory. Further, the neural correlates of episodic retrieval, measured by ERP amplitude and topography were not affected by ATD, leading the authors to suggest that processes other than retrieval (eg. encoding/consolidation) were impaired by reduction in serotonin synthesis.

If the age related decline in serotonin availability is a catalyst for memory deficits for older adults (Chen et al., 2000; Hendricksen, Thomas, Ferrier, Ince & O'Brien, 2004; Meneses, 1999; Michelsen, Prickaerts & Steinbusch, 2008; Wong et al., 1984) and given the parallel between recognition and source memory performance between older adults and younger adults during conditions of reduced serotonergic functioning, it is plausible that serotonin transmission assists in the binding processes required for source memory. Further, in a review of the serotonin and learning and memory literature in rodent and human studies, Buhot, Martin and Segu (2000) suggest that serotonin plays a crucial role in situations of increased cognitive demand, thus explaining why memory deficits under conditions of reduced serotonin functioning are often observed for more difficult tasks. This suggestion could potentially explain the intact recognition performance of older adults in the presence of a pronounced source memory deficit.

The reviewed research indicates that source memory is distinct from item memory and that part of this distinctiveness is the necessity to bind items together to form a unitary representation. This binding process requires additional processing than that of item memory and has been suggested to involve collaboration between the hippocampus and the frontal cortex. A number of studies have found disproportionate deficits in source memory compared to item memory in older adults, and this may be partially explained by a negative relationship between age and serotonin reserve. It could also be explained by reduced functional connectivity between these regions, reduced frontal functioning (Glisky et al., 2001) or cell atrophy in one or both of these regions. The age related deficit for source memory is not equal across tasks however, with two meta-analyses showing that the age related

impairments vary depending on the type of item to context binding required for successful task execution. This may be due to an increase in the distance between the to-be-remembered components, which increases the difficulty of the source task and therefore requires greater hippocampal involvement (Rugg et al., 2012; Starasina & Davachi, 2008).

The variability in age related findings for source memory performance prompted Spencer and Raz (1995) and Moscovitch (1992) to distinguish two different types of source memory tasks. Some elements of source information are intrinsic to the target items, and this form of contextual detail is referred to as *stimulus bound* (Spencer and Raz, 1995) or *associative* (Moscovitch, 1992). Examples of stimulus bound features include the medium in which content information is presented (such as the font colour of a target word or the gender of the voice it is presented in). Associative source information is more directly connected to the target item, and is therefore more easily encoded because the content and context are concurrently encoded. Spencer and Raz (1995) suggest that this is the reason why the magnitude of age differences in associative memory were small and equivalent to age differences in item memory. In contrast, other source information is extrinsic to the target items, and this form of source is referred to as *organisational* (Moscovitch, 1992) or *spatial temporal* (Spencer & Raz). Examples of organisational source features include memory for spatial location or temporal order, both tasks for which there was a greater magnitude in age deficits. This information is further removed from the target item and therefore encoding is more effortful as it requires greater strategic binding together of content and context. Some researchers explain age deficits for these organisational tasks as indicative of the higher demands placed on strategic frontal functions for successful task performance (Troyer, Winocur, Craik & Moscovitch, 1999).

According to the *Working with Memory model*, the hippocampal component is responsible for making associations among features within a discrete episode and binding these into a memory trace. The frontal component works with the hippocampus to engage in cognitive control processes that assist in encoding and retrieval. Moscovitch and Winocur (1992) suggest that source memory is a working

with memory task as it requires involvement of the frontal system to elaborate the link between the item and the context. They argue that due to the predominance of frontal lobe changes in older adults, and the pattern of results from the meta analyses indicating a particular disadvantage for older adults on organisational rather than associative source memory tasks, that associative tasks may require less involvement of the frontal component than organisational tasks. This model therefore offers an explanation as to why the Spencer and Raz (1995) and Naveh-Benjamin and Old (2008) meta-analyses reported larger effects for the tasks that required an item to associated with a location, which presumably requires more frontal and hippocampal involvement, than those tasks where the source is more intrinsic to the item, such as words presented in a particular colour or font. Thus, although it is clear from the reviewed literature that both systems are required for accurate source memory performance, the relative contribution of each region may vary as a consequence of binding requirements.

Associative learning deficits in regular consumers of ecstasy

The present experiment was designed to investigate whether source memory is impaired for people who take ecstasy, and if so, to assess whether this impairment is equivalent between an organisational and associative source memory task. Both older adults and ecstasy consumers have been reported to have a reduction in serotonin markers in various brain regions, and given the poor source memory performance of older compared to younger adults, it is plausible that ecstasy users may be deficient in the binding processes required for successful source memory performance. Previous research has investigated the effect of ecstasy use on paired associates tasks, which require participants to form a link between two or more previously unrelated items, and thus require the binding processes that are inherent to source memory tasks. For example, Montgomery, Fisk and Newcombe (2005) found an ecstasy related deficit on various associative learning measures, however for a measure of forgetting they reported that cannabis use accounted for poorer performance amongst ecstasy users. Gallagher et al., 2012 also reported poorer associative learning for ecstasy users relative to poly-drug and drug naïve controls, however a higher frequency of false positives for recent cannabis users was also evident. The clearest evidence of an associate learning deficit for ecstasy and not

cannabis users has been reported by Brown, McKone and Ward, (2010) who reported poorer performance for ecstasy users compared with drug naïve and cannabis using controls on a Verbal Triplet Associates task. This task consisted of a learning phase whereby the same list of word triplets (eg. frog-chair-apple) was presented to participants five times, so that they may learn the associations between the words. At test, they were presented with the first word (eg.frog) and were required to free recall the remaining two (chair and apple). Thus, they were required to use binding processes to learn the associations between words. Despite multiple list presentations, Brown et al., found that ecstasy users were unable to improve their performance on the verbal triplets task to the level of the drug naïve and cannabis-using control groups. To date, the limited literature on the effects of ecstasy on associative learning does not provide clear evidence for an ecstasy related impairment of the binding processes involved in executing source memory tasks.

The present study further examines the effects of ecstasy on associative learning by comparing ecstasy users and non-users on source memory performance. The aim of the study was to assess for differences in performance between ecstasy users and non-ecstasy users on two source memory tasks that vary in difficulty of the item to context binding requirements. Previous research has identified a disproportionate deficit between associative and organisation source memory tasks for older adults, who typically perform worse on the more challenging organisational source memory tasks (Old & Naveh-Benjamin, 2008; Spencer & Raz, 1995). For the present study, a Location and Colour source memory task have been chosen as the organization and associative tasks respectively. The reviewed literature on aging and source memory, and the *Working with Memory model* would predict that ecstasy users perform more poorly than the non-ecstasy users on the Location task compared to the Colour task, since both the hippocampal and frontal components are required for successful source memory performance.

Method

Participants

The present study was conducted at the same time as the list learning experiment reported in Chapter 7. The participants are described in the Method section of that chapter. Table 8.1 below provides a summary of the group characteristics. The groups were well matched for sex, age and estimated IQ and the differences in cannabis and ecstasy use were consistent with group selection criteria.

Table 8.1

Selected Characteristics of the Drug Groups in the Source Memory Study

	Drug naïve control (n=20)	Cannabis-only (n=15)	Ecstasy-only (n=16)	Ecstasy-plus-cannabis (n=17)	*F*	*p*
Sex (% female)	52.4%	40%	56.3%	47.1%	χ^2 = 0.94	0.81
Age (years)	25.3 (7.9)	24.2 (5.2)	22.9 (2.2)	21.2 (2.3)	2.1	0.11
General Intellectual Functioning (WTAR)	109.6 (11.1)	112.8 (11.2)	109.5 (8.7)	107.6 (12.3)	0.6	0.59
Ecstasy-Days used in past 6 months	0.00	0.00	13.69 (7.09)	16.12 (11.42)		
Cannabis-Days used in past 6 months [Δ]	0.00	39.07 (34.78)	0.38 (.71)	49.76 (36.79)	19.65	**<.001** EC, C > E, N

Source memory stimuli and apparatus

The stimuli used in both tasks consisted of 116 nouns, all six letters in length, selected from the Medical Research Council Psycholinguistic Database (Colthart, 1981, available online at http://www.psy.uwa.edu.au/mrcdatabase/uwa_mrc.htm). The words (in Appendix B) were of moderate frequency with an average Kucera-Francis (Kucera-Francis, 1967) frequency of 61, ranging from 40 – 100. The average Concreteness rating was 432, ranging from 275 – 600 (with 100 being low and 700 being high). These parameters were chosen to reduce the advantage for low frequency and highly concrete words in recognition tests (eg. Christian, Bickley, Tarka & Clayton, 1978; Gillund & Shiffrin, 1984; Gorman, 1961). From these words, six list blocks were created, matched for word frequency and concreteness; three for the Colour source task, and three for the Location source task.

The experimental tasks were created and run in Inquisit (Version 2.0). Task administration was computerised, using an IBM compatible computer with a 40 centimetre screen. All words were presented in Ariel, 36 point font. For the Location task, all words were presented in black. Viewing distance was approximately 65 cm. from the screen. The presentation order of the colour and location tasks was counterbalanced across participants within each group.

Colour task procedure (associative source memory task)

This task required participants to learn and recall the colour associated with a word stimulus. There were three stages to this task: Due to the complex nature of the response requirements, there was an initial response *practice* phase to allow participants to become familiar with where the response buttons were placed on the keyboard. During response practice a colour word (eg. "green") was presented in the middle of the screen in congruent coloured, 36 point, Ariel font. Participants were asked to respond by pressing the same coloured button as the colour on the screen. There were 18 trials, three for each colour and colour names were presented randomly for each participant.

Following response practice, the *encoding* phase began. During this phase of the task, words (non-colour words, e.g. "show") were presented one at a time, in a

different, pseudo random order on each trial (constrained so that words presented in the same colour were not displayed consecutively). Each word was presented once, in the middle of the screen in one of a possible six colours. Words remained on the screen for a duration of 2000 milliseconds, with an inter-stimulus interval of 250 milliseconds. Participants were instructed to memorise both aspects of the stimuli (the word and the colour it was presented in).

In the *recall* phase, the same words were presented individually in black font in the middle of the screen and participants were asked to think back to when they saw the word the first time, and indicate which colour it was presented in by pressing the corresponding coloured button on the keyboard. The words remained on screen until participants responded. There were three blocks, the first of which was designated practice, consisting of 18 trials each, making the highest score achievable 36. Participant responses (accuracy and reaction time) were recorded by the program.

Location task procedure (organisational source memory task)

The structure of the location task was the same as for the colour task. During response *practice*, the name of one of six locations (upper left, upper middle, upper right, lower left, lower middle, and lower right) appeared in the corresponding position on the screen, and participants were asked to press the corresponding button on the keyboard. There were 18 trials, three for each location and location names were presented randomly for each participant.

Following response practice, the *encoding* phase began. During the encoding phase of the task, words were presented one at a time, in a different, pseudo random order on each trial, so that words of the same location were not displayed consecutively. Each word was presented once only in one of six locations on the screen. Each word remained on the screen for duration of 2000 milliseconds, with an inter-stimulus interval of 250 milliseconds. Participants were instructed to memorise both aspects of the stimuli (the word and the location it was presented in).

In the *recall* phase, the same words were presented individually in black font in the middle of the screen and participants were asked to think back to when they saw the word the first time, and indicate which location it was presented in by pressing the button that corresponded to the location. There were six white buttons on the keyboard, the placement of which corresponded to the six locations the words were presented in (upper left, upper middle, upper right, lower left, lower middle and lower right. The words remained on screen until participants responded. There were three blocks, the first of which was designated practice, consisting of 18 trials each, making the highest score achievable 36. Participant responses (accuracy) were recorded by the program.

Design

The current study used a factorial design, with between-subject factors of Ecstasy-use (present, absent) and Cannabis-use (present, absent) and number of correct source judgments as the within-subjects factor.

Results

As expected, there was a significant effect of Task (F (1,65) = 12.35, p = .001, g = .53) with all participants making more correct source judgments on the Colour task (M = 19.02, SD = 5.17) compared with the Location task (M = 16.44, SD = 4.32).

There was no effect of Cannabis use, F (1,65) = 0.20, p = 0.660, however there was a significant effect of Ecstasy use (F (1,65) = 4.49, p = .038, g = -.50) on source judgements. Overall, the Ecstasy-present group made fewer correct source judgments (M = 16.20, SD = 1.03) compared with the Ecstasy-absent group (M = 19.26, SD = 6.03).

The Task × Ecstasy (F (1, 65) = .68, p = .412) and Task × Cannabis (F (1, 65) = 1.12, p = .294) interactions were not significant, however the Task × Cannabis x Ecstasy interaction approached significance (F (1,65) = 3.05, p = .085) and is presented in Figure 8.1. To clarify the interaction, separate between subjects analyses were conducted for the Colour task and for the Location task. For the Colour task, the effects of Ecstasy (F(1,65) = 2.03, p = 0.159, g = -.43) and Cannabis use (F (1,65) =0.67, p = 0.415, g = -.19) on source judgment accuracy were not

significant, and the Ecstasy x Cannabis interaction (F (1,65)=1.41, p = 0.239) was also non-significant.

For the Location task, the effect of Cannabis was not significant (F (1,65) = 0.01, p = 0.928) and Ecstasy and Cannabis were not involved in any interactions (F (1,65) = 0.11, p = 0.737). There was a significant, moderately sized effect of Ecstasy (F (1, 65) = 5.93, p = .018, g = -.57) however, with the Ecstasy-present group making significantly fewer correct source judgements than the Ecstasy-absent group.

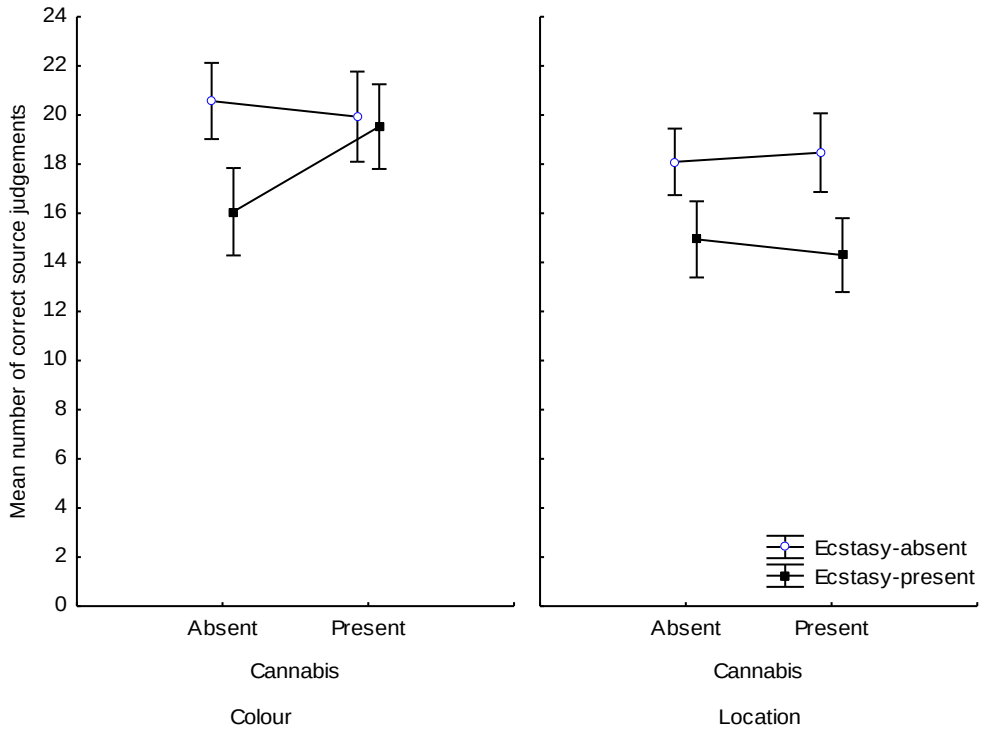

Figure 8.1. Mean number of correct source judgements on the Colour and Location tasks according to Ecstasy and Cannabis use (error bars represent standard errors).

Examining possible dose dependence effects

In order to evaluate whether there are dose dependent effects of drug use on source judgements, bivariate correlations were conducted between mean number of

correct source judgements for the Colour and Location source tasks and possible covariates, including cannabis, ecstasy and methamphetamine consumption, the correlations of which are reported in Table 8.2 below. The extent of cannabis use was not significantly correlated with either Colour or Location source judgements. Consistent with the Ecstasy effect for the Location but not Colour task data, ecstasy use was significantly correlated with Location source judgements, with greater frequency of ecstasy use in the preceding six months associated with lower location source accuracy. Although methamphetamine use in the previous six months was not significantly correlated with performance on either source memory task, there was a significant correlation between lifetime methamphetamine consumption and source accuracy for the Location task. Partial correlations revealed that when lifetime methamphetamine use was controlled for, the correlation between ecstasy use in the preceding six months and Location scores was no longer significant (r = -.16). There was a significant, positive correlation between lifetime methamphetamine use and ecstasy use in the preceding six months (r = .34, p <.01), which indicates that when controlling for methamphetamine use, the effect of ecstasy use on Location scores are also partially removed. Methamphetamine use may therefore be partially responsible for the poorer Location task performance for the ecstasy using group, although it does not rule out the co-contribution of the effect of ecstasy.
Further, lifetime methamphetamine consumption was entered as a covariate in ANCOVA but was not found to be a significant covariate of Location source judgments, thus the original, unadjusted, analyses are retained.

Table 8.2

Bivariate Correlations between Ecstasy, Cannabis and Methamphetamine use and Source Judgements.

	Colour Source Recall	Location Source Recall
Days used Ecstasy in last 6 months	-.13	-.26*
Lifetime occasions of Ecstasy use	.02	-.11
Days used Meth. in last 6 months	-.12	-.22
Lifetime occasions of Meth. Use	-.13	-.32**
Days used Cannabis in last 6 months	-.03	-.15
Lifetime occasions of Cannabis use	.05	.08
Colour source recall		.60**

Notes: * $p < .05$, ** $p < .01$

Discussion

The aim of the present study was to assess for differences in performance between ecstasy users and non-ecstasy users on two source memory tasks that vary in difficulty of the item to context binding requirements. In the Colour Task, the source (colour) was intrinsic to the item (the word) and this task is referred to as *associative* (Moscovitch, 1992) whereas in the Location task, referred to as *organisational*, the word was presented in a particular location on the screen, and thus the source is removed from the item to a greater extent that the colour task. Previous research has identified a disproportionate deficit between these tasks for older adults, who typically perform worse on organisational compared to associative tasks (Old & Naveh-Benjamin, 2008; Spencer & Raz, 1995).

Summary of the present findings

Source accuracy was significantly reduced in the Location task compared to the Colour task, and this was evident for all drug using groups. This pattern of results is commonly reported in the source memory literature and confirms that the organisational (location) task was more difficult to execute than the stimulus bound (colour) task, supporting the efficacy of the experimental design. The effect size indicated that on average, participants performed half a standard deviation lower on the Location task, which is a moderate magnitude effect.

The between-groups comparison failed to reveal any effect of Cannabis, indicating that in the present sample, cannabis use did not affect source memory performance. The overall analysis showed a detrimental effect of ecstasy use on source memory performance, although there was a trend ($p = 0.08$) for an Ecstasy x Cannabis x Task interaction. When separate analyses were conducted for each task, there was a moderate magnitude ($g = -0.43$), non-significant effect of ecstasy for the colour task and a larger magnitude ($g = -0.57$), statistically significant deficit for ecstasy users on the location task. This result mirrors that of the source memory and aging meta-analyses, with both ecstasy users and older adults performing worse on the organisational compared with the associative task. The absence of a Cannabis

main effect and an Ecstasy x Cannabis interaction suggests that this effect was apparent for ecstasy users regardless of whether they used cannabis or not, indicating that this result is not related to cannabis use.

Unexpectedly, results from the current study revealed that when lifetime methamphetamine use was controlled for, the negative relationship between ecstasy use in the preceding six months and Location scores was no longer significant. However, as there was also a significant relationship between ecstasy use in the preceding six months and lifetime methamphetamine use (participants who used more ecstasy in the last six months also had higher levels of lifetime methamphetamine use (r = .34) the partial correlations are not conclusive. Nevertheless, the possibility that higher rates of lifetime methamphetamine use are associated with poor source memory cannot be completely dismissed, and the cross-sectional design of the current study cannot attribute any causality to either ecstasy or methamphetamine use.

The present study has shown for the first time an ecstasy related deficit on source memory performance. Previous research has investigated the effect of ecstasy use on the broadly related methodology of paired associates tasks. These tasks require participants to form a link between two or more previously unrelated items, and thus require the binding processes that are inherent to source memory tasks. Consistent with the present study, ecstasy users have been reported to be impaired on various measures of verbal associates tasks (Brown, McKone & Ward, 2012; Gallagher, et al., 2012; Fisk, Montgomery, Wareing & Murphy, 2006; Montgomery, Fisk & Newcombe, 2005) although cannabis effects were also associated with higher rates of forgetting (Montgomery et al., 2005) and a greater number of false positives (Gallagher et al., 2012). In the present study, the potentially confounding effect of cannabis was controlled for by including a Cannabis group with very limited poly-substance use, demonstrating no group effects of Cannabis for source memory and no significant relationships between cannabis use and source memory.

Source memory deficits are associated with alterations in prefrontal and hippocampal functioning

These results have demonstrated some similarities between regular consumers of ecstasy and older adults with regard to their source memory performance. The meta-analyses by Spencer and Raz (1995) and Old and Naveh-Benjamin (2008) showed older adults performing more poorly overall, as well as having impairments of a smaller magnitude on the easier, associative tasks, such as judging whether a word was seen or heard (d = 0.71, 95%CI: 0.49 – 0.94), and larger magnitude effects for older compared to younger adults on the more difficult organisational (Location) source memory tasks (e.g. d = 1.01, 95%CI: 0.89 – 1.13). In the present study, ecstasy users also had poorer source memory performance compared with non-users, however separate task analyses showed an effect size that was small in magnitude, though non-significant for an ecstasy related deficit on the associative task (d = -0.43), and a significant deficit of moderate magnitude for the organisational task compared with cannabis users and drug naïve controls (d = -0.57). As such, the trends apparent in both the ageing and ecstasy consumer data were consistent, with both older adults and ecstasy users showing more pronounced source memory deficits than control subjects on tasks that place greater demands on binding processes. Further, inspection of Table 8 shows that the age of the Ecstasy-only group trends towards being lower than the age of the Drug naïve group. Despite their younger age however, the ecstasy using group are still performing worse than the older, non-ecstasy users. These findings raise the possibility that there are structural, functional and neurochemical similarities between regular consumers of ecstasy and older aged healthy adults.

The prefrontal cortex has been posited as one of the structures underlying source memory performance and source deficits have been shown to be related to frontal lobe damage or impaired executive functioning (eg. Ciaramelli & Spaniol, 2008; Duarte, Ranganath, Knight, 2005; Johnson, O'Connor, & Cantor, 1997; Schacter, Harbluk, & McLachlan, 1984; Shimamura & Squire, 1987; Simons, Verfaellie, Galton, Miller, Hodges, & Graham, 2002) and neuroimaging has shown increased activity in prefrontal areas during encoding or retrieval of source

information (Duarte, Henson & Graham, 2011; Hayes, Ryan, Schnyer, & Nadel, 2004; Mitchell, Raye, Johnson & Greene, 2006; Rugg, Fletcher, Chua & Dolan, 1999; Van Petten, Senkfor, & Newberg, 2000). Impaired frontal functioning has also been indicated for regular consumers of ecstasy, who have been demonstrated to have deficits in some executive functions (eg. Montgomery, Fisk, Newcombe & Murphy, 2005; Murphy, Wareing, Fisk & Montgomery, 2009; Smith, Tivarus, Campbell, Hilier & Beversdorf, 2006; Reay, Hamilton, Kennedy & Scholey, 2006) and markers of general neuronal loss in the frontal cortex of regular ecstasy users have been significantly correlated with previous exposure to ecstasy (Reneman et al., 2002). Further, alterations to neural activity in the dorsolateral prefrontal cortex have also been reported for regular ecstasy users (Bosch et al., 2013; de Win et al., 2007; McCann et al., 2008; Moreno-Lopez et al. 2012.Similarly, age related neuropathology has been demonstrated for the prefrontal cortex (Craik, Morris, Morris, & Loewen, 1990; Dennis & Cabeza, 2008; Raz, 2000; Raz & Rodrigue, 2006; West, 1996). Thus, it is possible that ecstasy users, like older adults have larger source memory impairments for organisational source tasks that require greater executive functions due to structural or functional changes to the prefrontal cortex.

The other identified structure involved in source memory execution are the medial temporal lobes, and in particular the hippocampus, which has been suggested as the structure in which two or more elements of an event are associated or bound together to form a memory. For this reason, the hippocampus is considered an important structure for the formation of source memories, damage to the hippocampus results in significant source memory deficits (eg. Schwert & Dopkins, 2001; Thaiss & Petrides, 2003) and neuroimaging data has also indicated increased activity in the hippocampus during encoding or retrieval of source information (Cansino, Maquet, Dolann & Rugg, 2002; Davachi, Mitchell & Wagner, 2003; Diana, Yonelinas & Ranganath, 2007; Dobbins, Rice Wagner & Schacter, 2003; Giovanello, Schnyer, & Verfaellie, 2009; Kensinger & Schacter, 2006; Mayes, Montaldi & Migo, 2007; Preston, Schrager, Dudukovnic & Gabrieli, 2004; Skinner & Fernandes, 2007; Starasina & Davachi, 2008). It has been suggested by various

authors that the memory impairments reported for ecstasy users may be due to functional changes to the hippocampus (Becker et al., 2013; Daumann et al., 2005; Fox et al., 2002; Gouzoulis-Mayfrank & Daumann 2009) Further, PET studies of regular ecstasy users have shown reductions in hippocampal volume (den Hollander et al., 2011) and reduced serotonin receptor binding in the hippocampus (eg. Kish et al., 2010). Likewise, age related neural changes have also been demonstrated in the medial temporal lobes (Driscoll et al., 2003; Giorgio et al., 2010; Raz, 2000; Raz et al., 2005).

Is there a role for serotonin in source memory deficits for ecstasy users?

Both older adults and regular users of ecstasy therefore appear to experience neural alterations either in the prefrontal or medial temporal cortices, and it is these changes that may be responsible for their respective source memory deficits. Although the cause of the underlying pathology for these deficits cannot be demonstrated in the present research, one commonality between regular ecstasy consumers and aging adults is reduced serotonergic functioning. The most robust finding from studies using acute tryptophan depletion has been lowered central serotonin levels impairs memory consolidation (Mendelsohn, Riedel & Sambeth, 2009; Scmitt, Wingen, Ramaekers, Evers & Riedel, 2006; Riedel, 2004). In normal aging, there is a reduction in serotonin transporter availability in the frontal cortex, hippocampus and diencephalon (Blin et al., 1993; Goldberg et al., 2004; McEntee & Crook, 1991; Meltzer et al., 1998; Meltzer, 1999; Meneses, 1999; Michelsen, Prickaerts & Steinbusch, 2008; Porter, Lunn & O'Brien, 2003; van Dyck et al., 2000; Verhoeff et al., 2000; Wong et al., 1984). More recently, decreases of around 10% per year have been reported for 5-HT_{1A} (Bhagwagar et al., 2004; Moller, Jakobsen & Gjedde, 2007) and 5-HT 1B (Matuskey et al., 2012) receptor binding in both the frontal and temporal cortices in healthy human subjects. Global reductions in SERT binding for people with Mild Cognitive Impairment compared with those age matched controls have also been reported (Hasselbalch et al., 2009: Rodriguez, Harun, Noristania & Verkhratsky (2012). As discussed in Chapter 3, reductions in SERT binding have been demonstrated for regular consumers of ecstasy and some studies have revealed an association between the degree of ecstasy use and reduction

in SERT levels (Buchert et al., 2003; McCann et al., 2005; Thomasius et al., 2003). These SERT reductions are sometimes of considerable magnitude, with Erritzoe et al. (2011) reporting SERT levels in medial inferior frontal cortex and superior frontal cortex to be 53% and 61% lower in ecstasy users compared with non-users, and SERT reductions were correlated with lifetime ecstasy consumption. Both the hippocampus and frontal cortex are innervated by serotonergic axons originating from the dorsal and medial raphe nuclei (Buhot, Martin & Segu, 2000) and serotonin pathways project from the hippocampus to prefrontal cortex (eg. Berumen, Rodriguez, Miledi & Garcia-Alocer, 2012). These regions and the connections between them are thus well placed for a role in learning and memory (Michelsen, Prickaerts & Steinbusch, 2008) and executive functions (Madsen et al., 2011). The present research indicates that ecstasy users have similar source memory problems to healthy older adults, and there appears to be other similarities also. For example, older adults and regular ecstasy users have both been demonstrated to have deficits of prospective memory (remembering to remember, for example, remembering to carry out an intended action in the future, eg. Heffernan, Jarvis, Rodgers, Scholey & Ling, 2001; Rendell, Gray, Henry & Tolan, 2007; Zakzanis, Young & Campbell, 2003, for a meta-analytic review of prospective memory and aging see Henry, MacLeod, Phillips & Crawford, 2004). Prospective memory is dependent on self-initiated retrieval processes associated with frontal function (Burgess, Scott & Frith, 2003; McFarland & Gilsky, 2009; Zakzanis et al., 2003). Ecstasy users have been demonstrated to be poor at self-initiating organisational strategies that may assist with encoding and retrieval (eg. Brown, McKone & Ward, 2010, reported poor semantic clustering for ecstasy users and this result was supported by the findings in Study 2) and collaboratively, deficits in source monitoring, organisational strategies and prospective memory are consistent with Craik's (1994) *self-initiated processing hypothesis* which explains age related memory decline as a function of the degree of effortful and strategic processes required for successful performance (Craik & Salthouse, 2000). The possibility that alterations to serotonergic functioning are compromising prefrontal and hippocampal functionality in ecstasy consumers, in a similar manner to that of older adults, is one that could be further explored.

Source memory deficits for ecstasy users are consistent with impaired frontal and hippocampal components of the Working with Memory model

According to the *Working with Memory model* (Moscovitch, 1992) the medial temporal component works in a relatively automated manner to bind events together and form a retrievable memory trace. This process is essentially automatic for information that is consciously attended to. The frontal component however, is strategic in that it is an effortful, self-initiated component that performs operations on the memory trace to assist with encoding and retrieval. The frontal component is not involved in the storage of information, but rather is involved in the "online" monitoring of input at encoding, and strategic retrieval processes, both of which work with the MTL to enhance memory performance. This model would therefore predict that associative source memory deficits arise from hippocampal dysfunction, and strategic source memory deficits would arise as a consequence of disproportionate impaired function of the frontal, relative to the hippocampal component. The Ecstasy x Task interaction apparent in the current study appears to support this view; the finding that there was a small, non-significant ecstasy related effect for the Colour tasks suggests that in this associative task, the hippocampal component effectively bound the colour and the word concurrently, so that when the word was presented during recall this provided a sufficient cue for all drug groups to correctly recall the colour. For the Location task however, the word cue at recall was more distal to the context (screen location) and was not sufficient to enable retrieval, therefore the frontal component needed to initiate a search process to find an associative cue to recover an appropriate memory. There are multiple and reciprocal connections between the prefrontal and medial temporal cortices (Remper-Clower & Barbas, 2000; Simons & Spiers, 2003) and since both these areas are involved in the formation of episodic memories (eg. Cansino, Maquet, Dolan & Rugg, 2002; Dobbins, Rice, Wagner & Schacter, 2003; Lucas & Ranganath, 2010; Simons & Spiers, 2003) it seems plausible that more complicated strategic source tasks are dependent on the integrity of both. Likewise, given ecstasy related dysfunction has been reported for both regions and that new evidence is emerging suggesting that functional connectivity between different cortical areas are impaired for ecstasy users (Karageougiou, 2011; Salomen, 2011) the *Working with Memory model* can

theoretically explain the regions most associated with source memory deficits for ecstasy users.

Is there a role for dopamine in impaired source memory?

Although there is some evidence to support the view that altered serotonergic functioning in the prefrontal and medial temporal cortices is the neuropathology that underlies source memory deficits in the current study, an alternative explanation should be considered, predominantly because of the finding of a significant relationship between lifetime methamphetamine use and Location task scores. Although MDMA causes release of dopamine acutely, its long term effects appear to be more selective to serotonin (Gudelsky & Yamamoto, 2008). Methamphetamine however, is a dopamine agonist which exerts its effects primarily via the dopaminergic system. Dopamine receptors are abundant in the prefrontal cortex and are believed to underlie some executive functions (Gonzalez-Burgos & Feria-Velasco, 2008) that are related to source memory retrieval (Piloino, Lamidey, Desgranges & Eustache, 2007). Executive dysfunction is sometimes reported for regular methamphetamine users (eg. Nestor, Ghahremani, Monterosso & London, 2011; Simon, Domier, Carnell, Brethen, Rawson & Ling, 2002) and PET studies have revealed reduced dopamine transporter densities (-33.3%) in the prefrontal cortex of regular methamphetamine users compared with drug naïve controls (Sekine et al., 2003) and decreases in DAT have also been reported for the orbitofrontal and dorsolateral prefrontal cortex (Sekine et al., 2006). Furthermore, in a review of acute phenylalanine/tyrosine depletion (APTD) studies (a monoamine challenge procedure like acute tryptophan depletion, which reduces central dopamine availability) Booij, Van de Boes and Riedel (2003) concluded that acute tryptophan and ATPD procedures affect different cognitive functions, with ATD impairing long term memory consolidation, and APTD primarily impairing working memory. Since methamphetamine use was correlated with Location scores, which are more dependent on the integrity of the prefrontal cortex, and not the Colour task, which may rely primarily on the hippocampus which is more closely associated with verbal learning and memory, it is possible that dopaminergic fluctuations in the prefrontal cortex have impacted on source memory performance in the strategic Location task.

Aside from the cross sectional study design of the present study, which cannot rule out premorbid differences between the different drug groups, there was also a lack of urinary analysis which means self-reported drug use was unable to be verified (although the self-report method has been shown to have sufficient reliability and validity (Darke, 1998; Harrison, Martin, Enev, & Harrington, 2007). A further limitation of the current study is the assumption that the particular source memory tasks employed do in fact correspond to the brain areas that are posited by the *Working with Memory* model to support associative and strategic source memory tasks, although the neuroimaging and lesion data do support roles for both the hippocampus and prefrontal cortex in these tasks. Similarly, as the tasks used were specific to this study, their validity with regard to measuring source memory, and not episodic memory in general, is untested. Nevertheless, the finding that participants performed more poorly on the Location, compared with the Colour task, and that ecstasy use was associated with poorer performance on the more difficult task lends some support for construct validity. With regard to future research, in addition to assessing source memory using a prospective study design, it may be useful to compare regular ecstasy users with healthy older adults and aged matched drug naïve persons on source memory performance to ascertain whether there are similarities in source monitoring deficits.

In conclusion, the present study demonstrated significant source memory deficits for ecstasy users compared with cannabis users and drug naïve controls. The effect of ecstasy was larger for the more difficult organisational source task compared with the associative task, and this result mimics findings on the effects of healthy ageing on source memory. It was suggested that the source memory deficits for ecstasy users and older adults share similar underlying neuropathology, most likely reduced serotonergic transmission in the prefrontal and medial temporal cortices. These results are consistent with those of the previous two studies, which have showed ecstasy related impairments on verbal episodic memory as well as strategic processes associated with the frontal cortex.

Chapter 9
Summary of Findings, Implications, Limitations and Directions for Future Research

Summary of aims and findings across studies

The studies reported in this thesis had three main aims. The first was to clarify which measure of verbal learning and memory, Trial 1, Total recall or Delayed recall, was most affected by ecstasy use. Narrowing down verbal memory deficits into these three measures allows greater specificity with regard to which cognitive processes may be impaired or spared as a consequence of ecstasy use. This was achieved by performing a meta-analysis of 23 studies that had reported data from list learning tasks for regular ecstasy users compared with non-users. The second aim was to further examine the components of memory that were most affected by ecstasy use, and to evaluate the respective roles of the medial temporal and frontal lobes in the pattern of memory performance observed for regular ecstasy users compared with non-users. This was achieved by engaging the *Working with Memory* model as a framework from which to interpret the list learning and source memory data. Of particular interest was whether the memory impairments reported for ecstasy users were purely *memory* related, or whether they were also associated with poor use of memory support processes, such as initiating strategies for encoding and retrieval. The final broad aim of this thesis was to investigate whether any observed differences between drug groups could be attributed to ecstasy or poly-drug use. This aim was largely achieved by including Ecstasy-only, Cannabis-only, Ecstasy-plus cannabis and a Drug naïve group in the design, thus allowing the independent and interactive effects of ecstasy and cannabis to be analysed.

Numerous studies have employed the CVLT or RAVLT to investigate memory ability for ecstasy users, however until now there has been no evaluation of which measure: Trial 1, Total or Delayed recall was most affected by regular ecstasy use. The meta-analysis in Chapter 6 demonstrated that ecstasy use does not impact on these three measures equally, with only a trivial effect of ecstasy use (g = -.15) found for Trial 1 scores. Consistent with this finding, the list learning studies

presented in Chapter 7 reported only small *(g* = -.36) to moderate (*g* = -.49) effects of ecstasy use on Trial 1 performance on the unrelated and related lists respectively, and both the meta-analysis and list analyses failed to identify a significant dose dependent relationship between ecstasy consumption and Trial 1 scores. Consistent with the small effect of ecstasy on Trial 1, there was no effect of ecstasy use on word span scores (*g* = .09) and word span performance did not significantly differ between groups. It is thus apparent that ecstasy use spares memory for a single presentation of words.

The ecstasy related effect becomes apparent for multi-trial free recall however, with the meta-analysis showing a moderate magnitude ecstasy related deficit on this measure (*g* = -.71) which remained at this magnitude even with the exclusion of drug naïve comparison groups. Again the results from the list learning study were consistent with the meta-analysis; ecstasy use had a large magnitude effect for unrelated (*g* = -.92) and related (*g* = -1.10) list types. Furthermore, Total and Delayed recall scores and ecstasy consumption were associated with a significant dose related response for the list learning tasks. However, there was no ecstasy dose-related effect on Total recall performance identified in the meta-analysis; this may be reflective of inconsistencies in the way in which ecstasy use is quantified which make it difficult for a consistent dose related effect to emerge between studies. In keeping with the significant ecstasy related deficits for verbal episodic memory, a more demanding test of episodic memory, *source memory*, was also impaired for regular ecstasy users. Though non-significant, there was a small ecstasy related effect for the easier, associative Colour task (*g* = -.43) and a moderate, statistically significant effect of ecstasy use for the more difficult, organisational, Location task (*g* = -.57).

A specific role for poly-drug use in impairments to long-term memory consolidation

It has been suggested that the memory deficits reported for ecstasy users are actually a consequence of poly-drug use (eg. Croft et al., 2001; Halpern et al., 2011; Hanson & Luciana, 2010). The findings from the series of studies in the current thesis however are not consistent with this, and demonstrated that regardless of the

extent of cannabis use, if a participant had used ecstasy they were more likely to have a memory deficit than if they had been ecstasy-naïve. Furthermore, many of the ecstasy related memory impairments were accompanied by effect sizes of moderate to large magnitude.

The only meaningful exception to this was Delayed recall. On the unrelated list, there was a very large effect of ecstasy on Delayed recall (g = -1.02) however for the more specific measure of lost access, which indicates the number of specific words that were recalled on the final learning trial (Trial 8) and not recalled at the delayed recall condition, an effect of both cannabis and ecstasy use was apparent. Also during Delayed recall, poly-drug use was associated with lower subjective clustering, although this was not the case for cannabis use during the learning trials. In contrast, Ecstasy use was associated with greater lost access and lower subjective clustering throughout the learning trials and at Delayed recall. These results suggest that ecstasy use impairs subjective clustering within learning trials, and this reduced clustering effects both short, inter-trial consolidation (measured by lost access) and long term consolidation (delayed recall) . Alternatively, cannabis users' ability to subjectively organise words to assist recall was only impaired during the 25 minute delay, and subsequently they lost more words in this condition only. This poly-drug effect for Delayed recall may explain why the meta-analysis showed the effect size for this measure reduced considerably when the drug naïve comparisons were excluded (from g = -.98 to g = -.60).

To summarise, poly-substance use may impact on Delayed recall scores for ecstasy users, indicating that poly-drug use remains an important factor to consider when identifying cognitive processes that may be selectively impaired by ecstasy use. However this was the only memory component that was associated with any substantial poly-drug effect, with several other components of memory showing clear deficits associated with moderate to large magnitude ecstasy effects. Furthermore, the inclusion of the Ecstasy-only group, who reported lifetime ecstasy use twice as high as that of Halpern et al.'s 2010 study, challenges their conclusion

that poly-drug use, rather than ecstasy use is driving cognitive deficits reported for ecstasy users.

The contribution of strategic and consolidation processes in verbal and source memory deficits for ecstasy users

The findings from the current thesis consistently implicate processing deficits associated with an interaction between prefrontal and medial temporal brain regions. The lower word recall for ecstasy users on both related and unrelated word lists indicate deficits in consolidation and retrieval, which are associated with hippocampal dysfunction. For these deficits to be attributed purely to impaired hippocampal functioning however, it would *not* be expected that deficient organisational processes such as semantic or subjective clustering would also be present. Indeed, if ecstasy use primarily impaired hippocampal memory processes, it could be anticipated that there would be little difference between ecstasy and comparison groups on measures of semantic and subjective organisation. This was not the case, with ecstasy users performing poorly on memory measures *and* frontal-strategic organisational indices, on both list types. The moderate to large magnitude effects for ecstasy use on the clustering indices suggest that *deficient strategising is having an additive effect on poor inter-trial consolidation among the ecstasy consuming group*. The findings of the source memory study are consistent with this interpretation. According to the *Working with Memory* Model, the item-to-source binding required in the Colour task would be a primary function of the hippocampus, the structure often postulated as the site for memory binding. This is because in the Colour source task, a word was presented in red for example, and participants were required to recall the colour of the word. Thus, the source was intrinsic to the item, and the strategising and organisational requirements were relatively low. Conversely, in the Location task, the words were presented in a specific location on the screen, the spatial location of a word was more *extrinsic*, or further removed from the item, and consequently there was a higher degree of organisation required from the prefrontal system to implement a strategy to assist the hippocampus to bind the item and source for correct recall. Thus the finding that ecstasy use did not significantly impair Colour task performance, but was associated with a moderate magnitude

deficit on the Location task again indicates memory deficits for ecstasy users are more likely to be found on tasks that require both hippocampal and prefrontal networks.

This conclusion in itself is not novel, with previous authors also arguing that the frequently reported verbal memory deficits for ecstasy users are a consequence of frontal and medial temporal interactions (Brown, McKone & Ward, 2010; Quednow et al., 2006). The present thesis has attempted to clarify the interaction between strategic, frontal processes and consolidation/memory processes required for remembering semantically related and unrelated words as well as recollection for source, and it is apparent that the lower level of strategic processing at encoding, whether in a list learning or source memory task, has contributed to poor inter-trial consolidation and impaired source accuracy respectively for ecstasy users. This finding is consistent with the previously reviewed evidence for ecstasy- related alterations in prefrontal (eg. Bosch et al., 2013; de Win et al., 2007; McCann et al., 2008) and hippocampal activity (Becker et al., 2013; Daumann et al., 2005; den Hollander et al., 2011).

Are the verbal memory deficits reported for ecstasy users associated with serotonin?

MDMA acutely increases the release and prevents reuptake of serotonin by SERT and at the residual level also inhibits tryptophan hydroxylase, an amino acid precursor of serotonin (Cadet, Jayanthi & Lyles, 2007). Since ascending serotonin neurons innervate the cortex and hippocampus, serotonin has emerged as an important neuromodulator of learning and memory (Ogren et al., 2008). Dopamine and noradrenaline are also modulated as a consequence of ecstasy consumption, however research has demonstrated that the sustained neural effects of ecstasy use impact the serotonergic system (Cadet et al., 2007). Although studies examining the impact of ecstasy use on the functioning of specific serotonin receptor subtypes is lacking, particularly with regard to the 5-HT_{1A} subtype, for which there is increasing evidence of a role in learning and memory (Cowen & Sherwood, 2013) there are several independent findings of reduced SERT density for regular ecstasy users in the hippocampus, cerebral cortex, including prefrontal areas and in the thalamus.

When considered in light of evidence that the magnitude of SERT function in regular ecstasy users is correlated with poorer performance on tests of verbal memory (Kish, et al., 2010; McCann et al., 2008) and that even prospective cohort studies have identified that for people who began to use ecstasy use within a twelve month period had significantly poorer scores on the RAVLT at the follow up test compared to people who had not used ecstasy (Schilt et al., 2007; Wagner et al., 2012) the abundance of research speculating a role for serotonergic disruptions to the CNS as the mechanism by which cognitive deficits for regular ecstasy users emerge appears justified.

Although the current thesis did not investigate the relationship between serotonin markers and memory performance for ecstasy users, it did evaluate the cognitive processes that underlie verbal memory deficits for this drug group, and found a specific effect of ecstasy use on short and long term *consolidation* and source memory *binding*. As serotonin receptors are involved in long term potentiation in the hippocampus (Berumen, Rodriguez, Miledi & Garcia-Alcocer, 2012) and the most consistent effect of ATD is impaired verbal memory consolidation (Mendelsohn, Riedel & Sambeth, 2009) and the structure demonstrated to support both consolidation and source memory binding is the hippocampus (Winocur & Moscovitch, 2011) the results from the present thesis are consistent with the hypothesis that alterations to serotonergic neural-transmission, particularly in the serotonin receptor-dense hippocampus, is a highly likely candidate for the impairment in consolidation and source memory binding among regular ecstasy users.

The present thesis also suggests that frontally mediated, strategic neural networks are not working effectively with the hippocampus to assist in encoding and consolidation among ecstasy users. Due to the recent neuroimaging studies that showed reduced functional connectivity between areas in the motor cortex and interactions between the thalamus and cortical areas for ecstasy users (Karageorgiou et al., 2009; Salomen et al., 2012) and noting the presence of the serotonergic pathways that project from the hippocampus to prefrontal cortex, this explanation

would be consistent with the view that there is a sustained down-regulation of serotonin and its markers (eg. SERT, Biezonski & Meyer, 2011; Kish, Fitzmaurice, Chang, Furukawa & Tong, 2008) among chronic ecstasy consumers, and this down-regulation interferes with serotonergic transmission between medial temporal and frontal regions. Thus the pattern of results in the current series of studies may arise as a consequence of faulty neural transmission between these regions, rather than simply as a consequence of localised regions of neural dysfunction.

An alternative view to the *Working with Memory*-model based explanation is that ecstasy use is associated with deficits on complex tasks that require efficient processing speed to maximise performance. This may provide a plausible explanation for performance on Trial 1, Recognition and the Colour source task being less affected by ecstasy use, as these tasks were all less cognitively demanding. This processing speed hypothesis could also account for why ecstasy users had poorer strategic engagement, as rapid processing would be required to quickly organise to be remembered items to enhance verbal recall. Thus, rather than the verbal memory deficits for ecstasy users reported in the current series of studies being attributed to impaired interaction between hippocampal and prefrontal activity, it might instead be the case that the deficits arise as a consequence of reduced global processing speed. This hypothesis was tested to some extent in Chapter 8 of the current thesis, by including a processing speed task (letter comparison) and controlling for processing speed statistically. There were no differences between groups on the letter comparison task however, and the effects of ecstasy use remained for the global list learning measures when processing speed was controlled for. The processing speed hypothesis has been considered previously, however differences in processing speed were unable to account for ecstasy users' performance on a working memory task (Waring, Fisk, Montgomery, Murphy & Chandler, 2007) and meta-analyses reveal considerably smaller magnitudes for processing speed when compared with verbal learning and memory (eg. Kalechstein et al., 2007; Zakzanis, Campbell & Jovanovski, 2007). Thus, the position that deficits amongst ecstasy users are a result of reduced processing speed cannot be supported at present.

Another potential mechanism by which the pattern of memory and strategic deficits observed for ecstasy users in the current thesis is cortisol. Extreme levels of high or low cortisol are associated with memory deficits: prolonged cortisol dysregulation is associated with reduced hippocampal volume (O'Hara et al., 2007) and source memory deficits (Mitchell & Johnson, 2009). Regular poly-substance and cannabis users show blunted cortisol release compared to controls during acute drug intoxication (eg. Carson et al., 2012; D'Souza et al., 2008) which could thus explain potential poly-substance effects on verbal memory performance. Ecstasy use has also been demonstrated to elevate cortisol levels acutely (eg. Lamers, 2003). Recently however, the cortisol hypothesis was tested by Kuypers, de la Torre, Farre, Pujadas and Ramaekers (2012) who administered MDMA either alone or with a cortisol synthesis inhibitor (metyrapone) to regular ecstasy + poly-drug users. They reported metyrapone successfully prevented the MDMA induced blood cortisol elevation, however MDMA dose still resulted in lower scores on a word learning task. Thus the reduced impact of (MDMA induced) cortisol elevation did not reduce the memory deficits, indicating that elevated cortisol levels associated with ecstasy use are not modulating memory function, at least during acute intoxication. This is the first study to directly assess the role of cortisol in memory dysfunction for ecstasy users however, and as such the role of cortisol in memory deficits remains inadequately tested. At present, the working with memory explanation, whereby memory deficits in ecstasy users arise as a consequence of impaired connections between frontal and hippocampal cognitive systems appears to be a more likely explanation than processing speed or cortisol related effects.

Practical and clinical implications

Over the eight learning trials of the unrelated list, the ecstasy group recalled an average of 22 words less than drug naïve controls, and recalled 15 fewer words over the five trials of the related list compared with drug naive controls. These deficits are significant and last beyond the acute phase of the ecstasy induced effects. According to the 2010 National Drug Strategy Household Survey, the best indicator of substance use in the general Australian community, 25% of males and 23% of

females aged between 20 and 29 had used ecstasy in their lifetime. As such, memory deficits associated with its use may have practical implications for those young adults who are undertaking vocational based or university level study, since assessment usually involves written tests which require adequate verbal memory skills. Chapter 7 showed however, that by Trial 8 of the unrelated list, ecstasy users were able to recall approximately the same number of words as the control participants at Trial 5. It will therefore be useful for regular ecstasy users to be aware of memory deficits arising from ecstasy use so as to allow themselves additional learning time for exams and maximising the likelihood of being able to pursue vocational interests.

In Australia, regular ecstasy users rarely seek treatment for drug related problems (Matthews & Bruno, 2010; Topp, Hando, Dillon, Roche & Solowij, 1999) although a small percentage may present to health professionals with symptoms of low mood (Matthews & Bruno). It will therefore be useful for health professionals to ensure that ecstasy use is included as part of their initial alcohol and other drug use assessment for clients, particularly those presenting with low mood or anxiety. Health professionals could subsequently provide psycho-education about ecstasy-related residual effects so that should these symptoms occur, ecstasy consumers recognise them as being drug related and take appropriate action. Targeted assessment of memory deficits will also be useful, as simple memory screens such as Digit Span Forwards, part of the Wechsler Adult Intelligence Test, which is correlated with Trial 1 performance (Lezak, 1995) and the Montreal Cognitive Assessment for Mild Cognitive Impairment (Nasreddine et al., 2005) which is a commonly used screening tool in the alcohol and other drugs sector will not capture the short term consolidation component that is particularly impaired by ecstasy use. Ideally, automated computerised memory assessments which can be quickly administered and scored and can accurately detect subtle impairments in strategic and consolidation memory processes could be developed for an ecstasy using population. The measure of lost access in the current thesis for example, could be automatically scored by a computerised test.

For the majority of ecstasy users who do not seek treatment, information on harm reduction practices that could reduce the neural harms associated with ecstasy use needs to be delivered via social media and consumer appropriate forums. These harm minimisation practices include; obtaining ecstasy from a reliable source, limiting the number of pills taken per session, spacing occasions of use so as to allow the CNS to recover and to prevent tolerance and subsequent increases in self dosing, and avoiding using other serotonergic agonists (such as SSRIs) during ecstasy use (Winstock, Marsden & Mitcheson, 2010) Other factors that may be helpful in reducing neurobiological harm include not using other stimulants during ecstasy use, taking regular breaks from dancing, avoiding dehydration and getting too hot, ensuring adequate sleep and nutrition for the days following ecstasy use (Parrott, 2005). In addition to providing information on these practices, it will be useful for the clinician to ascertain whether their client is concerned about potential memory problems, and if so, to work with the consumer to develop personalised memory strategies to assist recall. Studies that have administered the CVLT to recreational ecstasy users have reported that when a category cue is provided at recall, ecstasy users' recall scores do not differ significantly from control participants (Brown, McKone & Ward, 2010; Medina et al., 2005). The current thesis found that ecstasy users' were less impaired at subjective, rather than semantic organisation, therefore ecstasy users could be encouraged to organise the to-be recalled information in a manner that is subjectively meaningful to them, and to practice retrieving the information based on their own subjective cues. This strategy could also be used in conjunction with standard memory enhancing strategies such as chunking large pieces of information into smaller units and using acronyms as cues, using imagery and repetition. Further, computer assisted cognitive rehabilitation has been indicated to be beneficial to treatment seeking substance users when delivered in inpatient programs and this may be transferable to an outpatient setting (Vocci, 2008).

Limitations and directions for future research

As discussed in Chapter 6, the potential confounds that are inherent to cross-sectional studies in this field are well documented and as such, statements about

causation cannot be made. The current thesis did control for some potential confounds however, by matching participants on estimated IQ, including participants with minimal exposure to illicit drugs other than cannabis, which was also controlled for by its inclusion as an independent variable. The participants from the current studies were all self-selecting and recruited from Hobart, Tasmania, which is a small city and as such, there may be features of this local sample that are not generalisable to the larger ecstasy using population. For example, the estimated verbal IQ scores ranged from 107 to 112, which are slightly higher than the UK norms for this age group (103). Also, the exclusion of several participants on the basis of high lifetime exposure to other illicit drugs decreased the sample size as did the exclusion of a small number of participants based on failing the clinical screening for abstinence prior to testing, although urine tests were not administered for verification. The current studies also relied on participants' self-reports of drug use, although this method has been demonstrated to have adequate reliability and validity (Darke, 1998; Harrison, Martin, Enev, & Harrington, 2007). At the time of testing, purity testing based on police seizures indicated the median purity was around 25% (Illicit Drug Data Report, 2008-2009) however whether the pills participants reported taking contained MDMA was not independently verified. Further, data on the environmental factors present during ecstasy use, and concomitant use of other illicit substances, were not obtained, and as interactions between ambient temperature, metabolic stress, concomitant drug use and memory deficits may occur, future, large scale prospective studies should systematically evaluate these factors. Also, although it is clear from the one-way ANOVAs that the groups differed in the expected direction on the drug use variables, specific comparisons examining the difference between ecstasy and cannabis users using a non-parametric test such as the Mann Whitney-U tests could have been conducted. Finally, while the study was not blinded, the presentation of word stimuli in the list learning tasks was automated and source judgement accuracy was recorded by the Inquisit program, however testing would ideally have been undertaken by a double blind procedure to prevent potential experimenter bias.

The current thesis drew a parallel between ecstasy users and older adults on source memory performance, and found that the larger effect of ecstasy for the more difficult organisational source task compared with the associative task was consistent with the pattern of results obtained for healthy, older adults on these types of tasks. However, if ecstasy users' memory deficits resembled those of older adults, the magnitude of the ecstasy effect for the organisational (Location) source task (g = -.57) should have been of larger magnitude than those from the list learning tasks (unrelated; g = -.92, related (g = -1.10) as older adults consistently show a larger deficit for item rather than source memory. It is possible, however, that these effects may reflect differences in difficulty levels between the tasks than inconsistency between older adults and regular ecstasy users, because in order to reduce the demands on recognition/retrieval processes and focus on the recollection of the source, the source memory tasks did not include a recognition test in the recall phase. Instead all 18 words that were presented during encoding were re-presented at test, and participants were required to recollect one of six possible colours or locations in which the word was originally presented. This procedure may have reduced the difficulty of the source memory tasks, thus accounting for the smaller effect sizes seen for ecstasy users in this study in contrast to the effect anticipated if ecstasy produces effects on memory function akin to advanced ageing.

In order to examine this effect more thoroughly, it would have been advantageous to increase the number of words per trial from 18 to 24 to increase the number of word-source associations from 3 to 4. This limitation also highlights the need for future ecstasy and memory research to develop standardised assessments of cognitive functions, preferably based on neurocognitive concepts rather than using assessments designed for clinical populations which may be insensitive to subtle cognitive impairments. Nevertheless, the possibility that memory deficits for ecstasy users are similar to those observed for older adults is worthy of more attention, as in Australia, 24.1% of males and 19.8% of females aged between 30 and 39 have used ecstasy in their lifetime (with only 4.9% of this age bracket using in the past year: 2010 Australian National Drug Strategy Household Survey). If ecstasy use is associated with long term serotonergic disruption, in combination with decreased

brain serotonin levels with increased age (Bhagwagar et al., 2004; Moller, Jakobsen & Gjedde, 2007) this statistic potentially represents a health concern for aging ecstasy users.

It has previously been suggested that ecstasy users may share similar memory deficits to those of older adults (Morgan, 1998; Morgan, 2000; Wareing, Fisk, Montgomery, Murphy & Chandler, 2007) and this possibility has received some preliminary investigation (Schilt et al., 2009). Schilt et al. recruited a group of older ecstasy users (aged between 39 and 55) and compared their verbal memory effect size (d = 1.14) with younger ecstasy/poly-drug users (d = 1.06) from the Laws and Kokkalis (2007) meta-analysis. Schilt et al. noted that although the difference in effect sizes was small, they were in the expected direction and future prospective/longitudinal research will be better placed to differentiate between memory changes for older and younger ecstasy users. Information about the age of first use to determine whether early-onset ecstasy use is associated with altered development of the frontal cortex, as has been indicated for early onset cannabis use (Schweinsburg et al., 2008; Wilson et al., 2000) will be particularly useful.

In conclusion, findings from the studies in this thesis indicate that while impairments in verbal memory function are a robust residual effect of recreational ecstasy use, not all memory measures are affected equally, with more cognitively demanding tasks which require strategic engagement *and* adequate inter-trial consolidation being more susceptible to ecstasy related impairment than single trial measures. Memory deficits associated with ecstasy use are related to the level and type of strategic engagement applied during encoding, with greater ecstasy effects for semantic than subjective organisation. Short and long term consolidations were effected by ecstasy use, with a specific ecstasy related effect apparent for inter-trial consolidation, such that ecstasy users were impaired in their ability to consolidate previously recalled items. This poor consolidation was consistent with the reduced item to context source binding evident in the source memory tasks, and altogether this pattern of deficits are consistent with deficits to *both* strategic and consolidation processes. In contrast to the conclusions of Halpern et al. (2010) the present thesis

found consistent moderate to large magnitude ecstasy effects, which in addition to the degree of control over potential confounds such as verbal IQ, cannabis, alcohol and methamphetamine use, suggest that the memory deficits were related to ecstasy and not poly-drug use. The present thesis has identified consolidation as a process which is particularly vulnerable to the effects of ecstasy, and future research and clinical interventions may be able to design and administer assessments that are sensitive to this. Finally, in light of an aging population of ecstasy users, longitudinal research will be useful in tracking changes in memory across their lifespan.

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Appendix A

Word list stimuli

Unrelated list	**Recognition test**	**Related list**
sunburn	pliers	asparagus
oyster	referee	cabbage
pillow	twilight	beetroot
umbrella	harness	radish
locker	tornado	beetle
button	cement	mosquito
pollen	bishop	termite
salute	magician	spider
puzzle	pepper	canyon
timber	sofa	cliff
scissors	scholar	waterfall
umpire	duchess	glacier
daylight	pyramid	trombone
buckle	volcano	cello
hurricane	dungeon	clarinet
gravel	hurdle	violin
cardinal	portrait	linen
wizard	bacteria	velvet

paprika	chalk	suede
couch	frost	flannel

Appendix B

Source memory task stimuli

symbol	credit	estate	battle	weapon	sought
career	double	cousin	lawyer	impact	escape
honest	avenue	appeal	speech	leader	review
minute	mobile	happen	bright	slight	broken
travel	secret	dearly	decade	poetry	decide
animal	factor	screen	source	remove	author
finger	reduce	create	liquid	budget	weight
excess	cattle	unlike	garden	silent	regard
wisdom	highly	design	female	ballet	allies
target	narrow	desire	belief	lights	vision
lovely	annual	firmly	prison	column	booked
switch	useful	dollar	agency	memory	mental
remain	depend	nobody	crevice	danger	curled
notice	device	search	medium	spread	living
appeal	winter	proper	energy	active	matter
caught	notion	burden	accept	afraid	period
wooden	struck	smooth	league	golden	
flight	listen	tissue	beauty	marine	

detail	famous	master	junior	pocket
handle	wonder	glance	abroad	crisis